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2,794,786

THIONIN DYE-ION EXCHANGE RESIN INDICATOR COMPOUNDS

Harry L. Segal and Leon L. Miller, Rochester, N. Y.,
assignors to Security Trust Company of Rochester,
New York, as trustee for Harry L. Segal Medical Re-
search Fund

No Drawing. Application May 27, 1955,
Serial No. 511,786

10 Claims. (Cl. 260-2.2)

This application is a continuation-in-part of our earlier application Serial No. 146,384, filed February 25, 1950 (now abandoned), and our copending application Serial No. 212,698, filed February 24, 1951.

In our earlier applications there are disclosed novel non-toxic, resin ion exchange indicator compositions, administered orally, in which at least about 0.02 millimole of displaceable, identifiable indicator ion per dose (2 gm. dose) of resin exchanger is administered in unit dosage form for determining without intubation whether the pH of gastric juice in a stomach is above or below a predetermined pH value, thus providing a diagnostic indicator of particular value in clinical medicine.

In clinical diagnosis certain diseases such as pernicious anemia are associated with no free HCl in the stomach secretion. Carcinoma of the stomach is usually associated with very little or no free hydrochloric acid. Prior to this invention, it was necessary to subject the individual to the unpleasant procedure of introducing a tube into the stomach. The resin indicator compounds described here will obviate the necessity of the use of the intubation procedure which is not only unpleasant, but may also be difficult and even impossible under certain conditions.

The cure of gastric cancer depends on discovering the disease while it is still confined within the limits of the stomach. Since the disease is frequently silent, symptoms will not be of much aid even under the most enlightened circumstances. To apply routine mass fluoroscopy, G. I. X-ray series and conventional gastroscopy indiscriminately to all persons in the gastric cancer age group is impractical at present. Yet these diagnostic procedures are the only available means to discover the silent gastric cancer. The next best procedure might be the application of these available diagnostic techniques to a smaller group of individuals, who seem particularly liable to develop gastric cancer.

It is generally recognized that the incidence of gastric cancer is 3 to 4 times higher in people with achlorhydria than in the general population. The incidence may be ten times more frequent in the achlorhydrias with pernicious anemia. The incidence of achlorhydria in patients who have developed gastric cancer is 65% to 70%, as compared to that of only about 20% in the comparable age groups who are prone to develop carcinoma.

The ion exchange indicator compounds of this invention are prepared either by replacing the hydrogen cations of a carboxylic exchange resin with an equivalent number of an indicator cation or by adding an indicator anion to an anion exchange resin. The indicator cation or anion must be non-toxic in the dose employed, readily absorbed from the gastrointestinal tract after displacement from the compound and easily detectable in the body fluid chosen for its detection. The milliequivalents of the indicator ion displaced from a specific ion exchange indicator compound depends upon certain ion exchange properties such as the pH of the solution as well as the acid binding capacity of the ion exchange resin. The in-vitro estimation of the number of milliequivalents

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of the indicator ion eluted from a gram of compound by a specific pH of gastric secretion and by the various cations of small intestinal juice, plus the knowledge of the percent of such eluted ions to appear in the urine or blood collected during or after a definitive interval makes it possible to predict the dose and the range of gastric acidity detectable by each compound. Thus, this invention allows the determination of gastric acidity by the displacement of the identifiable indicator ions from the indicator resin compounds of this invention by the hydrogen ions of the HCl in the stomach. The indicator ions used are detectable by simple or relatively simple procedures.

This tubeless method has the important clinical advantage in that the diagnostic agent has no therapeutic or pharmacological effect on the body. The distress and alterations in the composition of gastric juice which may be experienced with the intubation technique are completely eliminated. This tubeless procedure may even be more accurate than the intubation technique for determining the specific range of gastric acidity detectable by the specific indicator ion exchange resin. Any discrepancy noted between the results of the intubation and tubeless techniques done on different days can be usually explained on a physiological basis. Gastric acidity may fluctuate for various reasons. In some cases, as already indicated, the mere act of intubation produces a cessation of gastric secretion. Reichsmann, Engel and Segal have found modification of the gastric secretory response to histamine during varying behavioral states in an infant with a gastric fistula. There is also a group whose gastric acid secretion may vary from no free acid to a low acid range.

Since the commercial ion exchange resin is a well-recognized material established by previous clinical and medical experience to be insoluble, inert and non-toxic in rats, dogs and human beings and since the indicator ion selected is also non-toxic in the amount employed in the unit dosage, the indicator compound formed therefrom is found to retain the desirable non-toxic properties of the separate ingredients in its diagnostic application.

Suitable ion exchange resin indicator compounds in accordance with this invention may be prepared from commercially available cation and anion exchange resins such as Amberlite IRC-50 (XE-96), a granular copolymer of methacrylic acid and divinyl benzene, Permutit H, a carboxylated phenol formaldehyde resin, and Permutit 70 or Amberlite IR-4B, a free synthetic phenolaldehyde resin and Amberlite IRA-400.

Thus, the ion exchange resin indicator compounds may be derived from either cation exchangers or anion exchangers depending upon the charge of the indicating ion which it is desired to incorporate into the ion exchange resin indicator compound.

The cation exchange resin such as a granular copolymer of methacrylic acid and divinyl benzene is conditioned, as described later, and then is treated with a solution containing a predetermined amount of the indicator ion in salt form. The resulting ion exchange resin indicator compound is removed from the solution, washed and dried, and thus prepared for use.

The displaceable indicator ions of the compounds of this invention may be selected from a group of dyes or other indicator ions which are known to be non-toxic and innocuous to humans in much larger doses than that used in these compounds. These cation or anion indicators are incorporated into the ion exchange resin indicator compound in quantitatively controlled amounts to provide the diagnostic indicator of this invention.

It has been discovered that the detection of the minimal quantity of gastric hydrochloric acid can be infallibly determined only if enough indicator cations are present

in the cation exchange resin indicator compound to be released in determinable amounts by the minimal quantity of hydrogen cations in the gastric juice. In testing for the presence of the minimal quantity in gastric juice excreted from a high acid stomach, the minimum quantity of indicator cation necessary in the ion exchange resin indicator compound may be as low as 0.02 millimole per dose of cation exchange resin indicator compound. In testing for the minimal quantity of gastric hydrochloric acid in gastric juice in stomachs having a low secretion of acid, the minimum quantity of indicator cation in the dose of cation exchange resin indicator compound will be proportionately higher. In every case, a quantity of 0.1 millimole of indicator cation in a dose of cation exchange resin indicator compound will be sufficient definitely to establish the presence of the minimal quantity of free gastric hydrochloric acid concentration.

The most important determination in an average gastric analysis is the estimation of the gastric acidity. The secretion of gastric hydrochloric acid by the parietal cells is constant. It is the volume not the concentration of parietal secretion, which varies and helps to determine the eventual concentration of the gastric acidity. Pure parietal secretion has an acidity of 165 meq./l. Yet the acid concentration of gastric contents usually is not above 60 C. U. (meq./l.). This is due to the changing proportion of parietal to non-parietal secretion as mucus, pepsin, etc.

The volume of gastric juice is generally parallel to the hydrochloric acid concentration determined therein. This is so because most of the water in gastric juice is secreted by the parietal cells. A high volume is generally accompanied by a high acid concentration. The volume, however, is not necessarily a direct measurement or indication of the acid concentration because duodenal juice, saliva and variations in mucus secretion affect the volume and thus indirectly the concentration of the hydrochloric acid.

Gastric acidity values are subdivided into four clinically significant ranges depending upon the meq./l. (clinical units) of HCl output; hyperacidity [50-100 meq. HCl/l. (pH 1.3-1.0)]; normal acidity [15-50 meq. HCl/l. (pH 1.8-1.3)]; low acidity [1-15 meq. HCl/l. (pH 3.5-1.8)]; achlorhydria [less than 1 meq. HCl/l. (pH 3.5-8.5)].

The displacement of the indicator cations from the indicator resin compound depends upon the hydrogen ion concentration (pH) as well as the quantity of the compound and the volume of solution to which the indicator compound is subjected. The average normal adult human stomach secretes about 2500 cc. of gastric juice in 24 hours under normal conditions. The range of secretion for a specific hour will depend upon certain conditions using appropriate stimulants such as caffeine sodium benzoate, histamine, etc. The average normal adult human stomach will secrete a volume of gastric juice ranging from 30 to 200 ml. averaging usually between 50 and 100 ml. Stomachs that secrete very little or no acid secrete on the average less than about 30 to 50 ml. of gastric juice in one hour after stimulation. Accordingly, on the basis of known acid secretion, an appropriate standardized unit has been predicted and administered to release a predetermined amount of indicator ion displaced by the hydrogen ions. The amount of these ions excreted in the urine will be an indication of the hydrogen ion concentration (pH) range which is characteristic of the indicator resin composition.

In vivo studies have shown us that the cation exchange resin indicator compound of this invention is administered best in a 2 gram dose for a given test of the presence of a minimal quantity of hydrogen cations in the gastric juice. This 2 gram dose is preferably made up of cation exchange indicator compounds containing preferably at least approximately 0.1 millimole of the indicator cation per dose of cation exchange resin compound and not less than 0.02 and not more than 0.50 millimole

of cation indicator per dose of cation exchange resin compound.

Particularly useful indicator ion components are dyes or such ions which can be detected by a color change and which conform to the requirements above mentioned, i. e. non-toxic, not too readily displaced by any cations other than those associated with the HCl of the stomach, readily absorbed and excreted in the urine. Such dyes or ions are detected quantitatively by colorimetry or by comparison with appropriate color standards. This makes it possible to determine the amount of dye or ion excreted in the urine, present in blood, etc. within a certain predetermined time after the oral administration of the indicator resin compound. Gastric secretion may be stimulated using standard reagents such as caffeine sodium benzoate, alcohol, histamine, etc. Conditions of the test are standardized to provide reproducible and accurate quantitative results.

Both the azo and quinone-imine group of dyes have been used as the indicator ions in preparing these compounds. Particular desirable dye indicator ions for colorimetric gastric acidity determinations, avoiding the inconvenience and disadvantage of intubation are certain of the basic dyes of the quinone-imine groups, the indamines. More specifically, the methylated amino derivatives of thiazins and their homologues which are substituted with two amino groups but the basicity of said amino groups being reduced by methyl substitution on the nitrogen atoms of said amino groups. These are symmetrical and asymmetrical methyl substituted thionins or their homologues free from solubilizing groups or acidic or basic reacting groups attached to the thionin nucleus. These basic dyes are blue, have a spectrophotometric absorption maxima in the range of about 610-670 millimicrons and are very well known as biological stains, see Conn, "Biological Stains," published by Biotech Publications, fifth edition, pages 90 through 95, 97 and 98.

These dyes are preferably used in the form of the chloride and include azure A, asymmetrical dimethyl thionin, azure C, monomethyl thionin, azure B, trimethyl thionin, Methylene Blue, tetramethyl thionin, as well as methyl homologues, such as Toluidine Blue O, Color Index No. 925. Also included are mixtures of these such as polychrome methylene blue, methylene azure, azure I and azure II, in which mixtures varying amounts of azure A and/or azure B are present.

The tetramethyl substituted thionin, methylene blue, which is typical of the entire class, is perhaps the best known non-toxic basic staining dye. Methylene blue is so readily oxidized that the presence of the azure (azure A), the lower methylated substitution products produced by such oxidation is universally recognized (see Conn). The presence of azure A in Stain Commission acceptable or medical grade USP methylene blue chloride, particularly where the methylene blue chloride solutions have been standing for some time accounts not only for some of the valuable staining properties of this preparation but also for improved gastric acidity determinations due to the presence of this material in the methylene blue ion exchange resin compounds of the present invention.

In order that this invention may be more fully understood, the following examples are given by way of illustration, but it is to be understood that the invention is not limited thereto as will be more specifically pointed out hereinafter.

EXAMPLE I

The cation resin Amberlite IRC-50 (XE-96) in the hydrogen form is treated with an aqueous solution of a suitable sodium salt or sodium hydroxide and then restored to the hydrogen cycle by eluting with hydrochloric or sulphuric acid. In the case of the methylene blue indicator exchange compound the cation exchange resin is conditioned as follows:

1. A measured amount of the cation exchange resin

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Amberlite XE-96 is placed in distilled water for 24 hours, using 100 grams of resin to 225 ml. of water.

2. The resin and water mixture is poured into a glass column at the bottom of which is a perforated porcelain plate covered with nylon. The water is drawn off to one-half inch above the resin. Two percent HCl is added and allowed to run through at the rate of 70 ml. per minute until the percolate has a pH of 2 or less.

3. The column is then washed with distilled water until the pH of the percolate rises to between a pH of 4-5.

4. One percent sodium hydroxide is added until the percolate reaches a pH of 10 or more.

5. The column is again washed with distilled water until the pH of the percolate is between 8.5-9.0.

6. Two percent HCl is added until the pH becomes 2 or less and then the column is washed with distilled water until the pH rises to between 2.5 and 3.5.

The resin is now conditioned and ready for the methylene blue indicator cations. "MB" will be used to indicate the tetramethylaminophenothiazinium ion. Using the batch process the methylene blue indicator compound is made as follows:

1. Methylene blue hydrochloride is dissolved in distilled water and added to the conditioned resin so that the desired amount of methylene blue cations are available for each gram of resin. This is stirred for six hours.

2. The solution is decanted off and the resin compound is thoroughly washed with distilled water until no more dye is present in the wash.

3. The resulting methylene blue exchange indicator compound is dried at room temperature. Each gram of this methylene blue exchange indicator compound contains the desired amount of methylene blue per gram of indicator compound.

We have prepared and tested methylene blue resin compounds containing 34.5 milligrams, 65.3 milligrams, and 68.5 milligrams per gram of resin.

These compounds were tested with dilute solutions of HCl as well as with Ringer's solution and alcohol. The amount of dye displaced by the hydrogen ions of the HCl depended upon the hydrogen ion concentration. An insignificant amount of dye is displaced at a pH above 3 and also by the cations in Ringer's solution.

EXAMPLE II

A cation exchange resin made up of a granular copolymer of methacrylic acid and divinyl benzene hereinafter referred to as Amberlite XE-96 is first conditioned and then treated with azure A ($C_{14}H_{14}N_3SCl$) (molecular weight 291.8), for the preparation of an azure A ion exchange compound of this invention. Azure A cations are substituted for the quininium or methylene blue cations in an amount that will allow one gram of the azure A indicator compound to contain the amount of azure A desired per gram of azure A indicator compound as already stated.

Compounds were made containing both 45 mg. and 58 mg. per gram of resin (weight on air-dry basis).

The azure A indicator compound (45 mg. of azure A per gram of resin) was then placed in dilute HCl solutions of varying hydrogen ion concentration as well as in Ringer's solution in the following manner: One-tenth gram of the azure A indicator compound was placed in each of 13 test tubes, to 11 of which were added 10 ml. of HCl solution of varying hydrogen ion concentration. To each of the remaining 2 test tubes 10 ml. of distilled water and of Ringer's solution were added, respectively. The temperature was kept constant at 37.5° C. and the mixtures were stirred for 30 minutes by a slow stream of air. The amount of azure A eluted was determined with a Beckmann colorimeter.

The following table reveals the amount of azure A that was eluted from one gram of the azure A indicator compound subjected to 100 ml. of each of these solutions:

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Table 1

pH of HCl Solution	Amount in mg. of Azure A cations displaced from 1 gram of compound, per 100 ml. of solution
5	5.7
8	8.2
10.1	8.0
1.50	13.4
1.89	8.0
2.81	4.25
2.72	1.65
3.00	0.91
3.20	0.71
3.62	0.87
4.00	0.10
H ₂ O	0.00
Ringer's solution	0.72

In preliminary clinical study the excretion time of the azure A dye was determined by measuring the amount of the dye excreted in urines obtained one hour and two hours following the oral administration of azure A in 20 or 40 mg. doses. The amount of azure A in the total two-hour excretion of 18 patients was found to vary from 7 to 26 percent with an average of approximately 15 percent. From this excretion factor and from the amount of azure A displaced in vitro by hydrochloric acid and by Ringer's solutions, a dose of 2 grams of the azure A resin compound was predicted as the most appropriate dose.

Definitive evaluation of the azure A indicator compound by us resulted from the clinical trial in 279 individuals at the established dose level of 2 grams. The gastric secretory response of 100 of these individuals to caffeine was determined by intubation. The gastric secretory response of the remaining 179 individuals was determined by the quininium resin indicator compound. On the trial day, breakfast was omitted. A glass of water containing 500 mgs. of caffeine sodium benzoate was administered to stimulate gastric secretion, and a control urine was collected one hour thereafter. The azure A indicator compound was administered in water and urines were collected one and two hours or only two hours thereafter. The amount of azure A present in the first and second hour excretions or in the total two-hour urine excretion was estimated both by the use of a Beckmann spectrophotometer at 620 millimicrons and by means of simple colorimetric comparator standards.

The interpretation of the results obtained with the azure A resin compound containing 45 mg. of azure A per gram of resin is summarized as follows:

Free Gastric HCl Secretion	Mg. of Azure A in 2-Hour Urine
Present.....	0.6 or more.
Absent.....	0.3 or less.
Borderline.....	0.3-0.6.

¹ This will depend upon the amount of azure A per gram of resin.

Table 2 is a summary of the results in the 279 individuals tested with azure A resin compounds. The five discrepancies can be explained on the physiological bases previously described.

Table 2
TUBELESS GASTRIC ANALYSES WITH AZURE A RESIN COMPOUND

Control Group	No. of Indiv.	Results	
		Consistent	Non-Consistent
Free Acid.....	208	206	2
Achlorhydria.....	57	55	2
Intermittent or Borderline Secretors.....	9	9	0
Sub-total Gastrectomy.....	5	4	1
Total.....	279	274	5

PROCEDURE OF THE TUBELESS GASTRIC ANALYSIS TECHNIQUE

In performing these tests, the following procedure is used: The individual takes no food after midnight. At a designated hour the next morning the individual urinates and saves this urine for a control. The individual then drinks a glass of water containing the gastric stimulant such as caffeine sodium benzoate. One hour after drinking the water with the stimulant, the individual urinates and saves the urine as a second control. He then takes the ion exchange resin indicator compound in about one-half glass of water. The dose is stirred vigorously and then is drunk immediately. The individual then urinates 1 and 2 or just 2 hours after consuming the dose. This or these urine specimens are tested for the amount of displaced indicator ions. The time of the test may be shortened by omitting the gastric stimulant. However, the standard test with the gastric stimulant must be repeated if the abbreviated test is negative for free acid.

The gastric acidity of the individuals who were the subjects of these tests was determined by removing samples of the gastric juice from each of the individuals by the usual intubation technique. The gastric acidity tests of the samples so obtained indicated that the subjects could be divided into a group in whom no free hydrochloric acid was present in the gastric juice and a group in whom there was free hydrochloric acid in the gastric juice. Each of the subjects was tested for free hydrochloric acid in the gastric juice by use of the ion exchange indicator compound of this invention.

Thus, as we have pointed out, this invention has provided for the determination of gastric acidity by the displacement of identifiable indicator ions from the indicator resin compounds by the hydrogen ions of the HCl of the stomach. The indicator ions are detectable by what we believe to be simple and effective procedures. Within the spirit of this disclosure we, therefore, claim as our invention the following:

1. A diagnostic indicator ion exchange compound for

determining without intubation whether the pH of gastric juice in a stomach is above or below a predetermined pH value, comprising a non-toxic, insoluble granulated cation exchange complex synthetic polymer resin containing from about 0.01 millimole to about 0.6 millimole of displaceable non-toxic thionin dye ions per gram of resin so as to be displaceable from the ion exchange resin predominantly by the ions from free hydrochloric acid secreted in the stomach, said dye ions being absorbed readily from the gastrointestinal tract and being easily detected by the dye color in urine or blood.

2. A compound as claimed in claim 1 wherein said dye ion is a cation from polychrome methylene blue.

3. A compound as claimed in claim 1 wherein said dye ion is the cation from azure A.

4. A compound as claimed in claim 1 wherein said dye ion is a cation from azure B.

5. A compound as in claim 2 wherein said resin is a copolymer of methacrylic acid and divinyl benzene.

6. A compound as in claim 3 wherein said resin is a copolymer of methacrylic acid and divinyl benzene.

7. A compound as in claim 4 wherein said resin is a copolymer of methacrylic acid and divinyl benzene.

8. A compound as in claim 1 wherein said anion exchange resin is a phenol aldehyde resin.

9. A compound as claimed in claim 1 wherein said dye cation is the cation from toluidine blue.

10. A compound as claimed in claim 1 wherein said dye cation is the cation from azure C.

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A Novel Family of Small Cysteine-rich Antimicrobial Peptides from Seed of *Impatiens balsamina* Is Derived from a Single Precursor Protein*

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Four closely related peptides were isolated from seed of *Impatiens balsamina* and were shown to be inhibitory to the growth of a range of fungi and bacteria, while not being cytotoxic to cultured human cells. The peptides, designated Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, are 20 amino acids long and are the smallest plant-derived antimicrobial peptides isolated to date. The Ib-AMPs (*I. balsamina* antimicrobial peptides) are highly basic and contain four cysteine residues which form two intramolecular disulfide bonds. Searches of protein data bases have failed to identify any proteins with significant homology to the peptides described here. Characterization of isolated cDNAs reveals that all four peptides are encoded within a single transcript. The predicted Ib-AMP precursor protein consists of a prepeptide followed by 6 mature peptide domains, each flanked by propeptide domains ranging from 16 to 35 amino acids in length. Such a primary structure with repeated alternating basic mature peptide domains and acidic propeptide domains has, to date, not been reported in plants.

An increasing number of cysteine-rich antifungal and antimicrobial peptides have been isolated from plants and in particular from plant seed. These peptides may have an important role to play in the protection of plants from microbial infection, and they could prove to be useful tools for the genetic engineering of fungal resistance in transgenic plants (1). Based on amino acid sequence homology, these peptides fall into at least six different classes. They include peptides isolated from seed of *Mirabilis jalapa* (1), *Amaranthus caudatus* (3), and *Zea mays* (4), members of the thionin family of peptides (5), members of the lipid transfer proteins (6–8), and members of the plant defensins (9–14).

From extracts of seed of *Impatiens balsamina*, we have isolated four small peptides the amino acid sequences of which are very closely related to each other but that do not resemble any peptides previously characterized from plants or other organisms. This paper describes the purification of the peptides and reports on their antimicrobial properties, in particular with

respect to the inhibition of the growth of plant pathogenic fungi. Furthermore, a single class of cDNA has been identified that encodes all four members of this family of peptides as part of a preproprotein. Details of the characterization of the unusual structure of this cDNA and its products, as well as of their expression patterns, are presented.

EXPERIMENTAL PROCEDURES

Biological Materials—Seeds of *I. balsamina* were purchased from Sandeman Seeds (Pulborough, United Kingdom). Fungi and bacteria were grown and maintained as described previously (14, 15). The following fungal strains were used: *Alternaria longipes* (CBS62083); *Botrytis cinerea* (K1147); *Cladosporium sphaerospermum* (K0791); *Colletotrichum gloeosporioides* (SR24BTA); *Fusarium culmorum* (K0311); *Gloeodes pomigena* (field isolate; T. Sutton); *Gloeosporium solani* (CBS19432); *Nectria galligena* (MUCL6128); *Penicillium digitatum* (K0879); *Phialophora malorum* (field isolate; D. Sugar); *Sclerotinia sclerotiorum* (SES A); *Trichoderma viride* (K1127); and *Verticillium albo-atrum* (K0937). The following Gram-positive bacterial strains were used: *Bacillus subtilis* (JHCC 55331); *Micrococcus luteus* (ATCC 9341); *Staphylococcus aureus* (ATCC 25923); *Streptococcus faecalis* (ATCC 29212); and the following Gram-negative bacterial strains: *Erwinia amylovora* (CFBP1430); *Escherichia coli* (HB101); *Proteus vulgaris* (JHCC 558711); *Pseudomonas solanacearum* (R48/a); *Xanthomonas campestris* pathovar *pelargonii* (INRA 10342); and *Xanthomonas oryzae* (ETH 698).

Extraction of Peptides—The purification of antimicrobial peptides from the basic protein fraction of *I. balsamina* seed was essentially as described previously (10). One-kilogram amounts of seed were ground in a coffee mill, and protein was extracted by stirring overnight at 4 °C in extraction buffer (10 mM Na₂HPO₄, 15 mM NaH₂PO₄, 100 mM KCl, 2 mM EDTA, pH 7). Ammonium sulfate was added to 80% relative saturation, and precipitated proteins were collected by centrifugation, resuspended in distilled water, and extensively dialyzed against distilled water using 2000-Da cutoff dialysis tubing (Sigma). The extract was adjusted to 50 mM NH₄Ac (pH 9) and passed over a Q-Sepharose Fast Flow column (12 × 5 cm, Pharmacia) equilibrated in 50 mM NH₄Ac (pH 9). The unbound fraction represents the basic protein fraction, and this was adjusted to pH 6 with acetic acid and passed over a S-Sepharose Fast Flow column (10 × 2.6 cm, Pharmacia) equilibrated in 50 mM NH₄Ac (pH 6). Bound proteins were eluted with a linear gradient of 50 mM–1.5 M NH₄Ac (pH 6) over 325 min at a flow rate of 3 ml/min. Proteins were monitored by the on-line measurement of the absorbance at 280 nm. Fractions with the highest antifungal activity were pooled for each peak and further purified by RP-HPLC¹ on a Pep-S column (C₁₈ silica, 25 × 0.93 cm, Pharmacia). Peptides were eluted with linear gradients of 0.1% (v/v) trifluoroacetic acid to 99.9% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid over 100 min at a flow rate of 3 ml/min. Elution of peptides was monitored by absorbance at 210 nm.

Electrophoresis and Amino Acid Sequencing—The purified peptides were analyzed by SDS-PAGE on precast high density gels (PhastSys-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) Y14369.

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¹ The abbreviations used are: RP-HPLC, reversed phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; AMP, antimicrobial peptide; Ib, *Impatiens balsamina*; SSPE, saline/sodium/phosphate/EDTA.

tem, Pharmacia), the sample buffer containing 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM EDTA, 0.005% (w/v) bromophenol blue, and 1% (w/v) dithiothreitol. Peptides were diffusion blotted onto 0.2- μ m pore size nitrocellulose (Schleicher & Schuell) and visualized by silver staining (16). For N-terminal amino acid sequence analysis, peptides were reduced and cysteine residues were alkylated with 4-vinylpyridine prior to digestion with either trypsin (EC 3.4.21.4) or chymotrypsin (EC 3.4.21.1), both from Promega. Following protease digestion, peptide fragments were separated by RP-HPLC (Pep-S column, C_{18} silica, 25 \times 0.93 cm, Pharmacia) and eluted with linear gradients of 0.1% (v/v) trifluoroacetic acid to 99.9% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid over 100 min at a flow rate of 3 ml/min. Peptide fragments were subjected to N-terminal sequencing by automated Edman degradation using a 477A Protein Sequencer (Applied Biosystems).

Mass Spectrometry—Matrix-assisted laser desorption/ionization-time of flight mass spectrometry was performed by M-Scan Ltd. (Ascot, UK) using a PerSeptive Biosystems VoyagerTM Elite BiospectrometryTM Research Station laser-desorption mass spectrometer coupled with Delayed ExtractionTM.

Antifungal and Antibacterial Assays—Antifungal and antibacterial assays were conducted as described previously (11, 14). The growth medium for the antifungal assays was either potato dextrose broth (Difco) at 12 g/liter (medium A), or medium A supplemented with 1 mM $CaCl_2$ and 50 mM KCl (medium B).

Antibacterial assays were carried out in 1% (w/v) tryptone (Sigma), 0.5% (w/v) low melting point agarose for *B. subtilis*, *Escherichia coli*, *M. luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Streptococcus faecalis* and in 1% (w/v) Bacto-Peptide (Difco), 0.5% (w/v) low melting point agarose for *Erwinia amylovora*, *Pseudomonas solanacearum*, *X. campestris*, and *X. oryzae*. Magainin I (Sigma) was used as a positive control in the antibacterial assays. Growth in each assay was assessed after the appropriate incubation period (48 h at 24 °C for fungi and 24 h at 28 °C for bacteria).

Human Cell Membrane Integrity Assay and Hemolytic Activity Assay—Membrane integrity of cultured human cells was tested on skin muscle diploid fibroblasts using the neutral red uptake method as described previously (10). Hemolytic activity was assayed using human blood group A erythrocytes as described previously (10) except that the erythrocytes were suspended in phosphate-buffered saline instead of 150 mM myo-inositol.

Construction and Screening of cDNA Library—Total RNA was purified from dry seed of *I. balsamina* by grinding in liquid nitrogen, extraction with phenol/cresol and phenol/chloroform, followed by lithium chloride precipitations (17). Poly(A)⁺ RNA was isolated by a Poly(A)Ttract mRNA Isolation System utilizing magnetic beads (Promega). cDNA was synthesized using a ZAP-cDNA synthesis kit (Stratagene) and, following the ligation of EcoRI linkers and size fractionation on a Sephacryl S-400 column (Pharmacia), a library constructed in λ -ZAP (Stratagene). A DNA probe for screening the cDNA library was generated by performing PCR on the above seed cDNA fractions using a pair of degenerate oligonucleotide PCR primers based on reverse translation of the available amino acid sequence of Ib-AMP1 peptide. The degenerate primers utilized were IbAMP1-C (5'-GTTG^T/_CTG^T/_CGGITGGGGIC-3') and IbAMP1-B (5'-CACCAIC^T/_GIAC^G/_ACA^G/_TA-3'), where I represents an inosine residue. The resulting 46-base pair PCR product was eluted from an acrylamide gel, purified using a Mermaid Kit (Bio101), and labeled with [α -³²P]dCTP using a Random Prime DNA Labeling Kit (Boehringer). The labeled PCR product was used to screen the cDNA library by plaque hybridization, following the transfer of near-confluent plaques to nylon membrane (Hybond-N, Amersham) and the UV cross-linking of DNA. Nonstringent hybridization was performed in 0.25% (w/v) Marvel (Premier Beverages, UK), 5 \times SSPE (1 \times SSPE is 0.15 M NaCl, 0.01 M NaH_2PO_4 , 0.001 M EDTA, pH 7.4), 0.1% (w/v) SDS at 35 °C and washing in 6 \times SSC followed by 3 \times SSC at 42 °C (1 \times SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). A total of 12 hybridizing plaques were purified and subsequently *in vivo* excised to generate subclones in pBluescript SK (-) phagemid vectors (Stratagene). Sequencing was performed using a Sequenase kit (United States Biochemical). Nucleotide sequence assembly and analysis was performed using PC/GENE and IG-SUITE Intelligenetics software.

Northern and Western Blot Analysis—Seed representing a range of developmental stages was harvested from glasshouse-grown plants. Developing seed was arbitrarily classified into 5 stages based on morphology and size: stage 1 = white seed, ~1 mm in diameter; stage 2 = pale green/white, 2 mm; stage 3 = pale green/white dry, 3 mm; stage 4 = gray, dry, 4 mm; and stage 5 = brown seed coat, 3–5 mm. For germination studies, seed were germinated on damp filter paper at

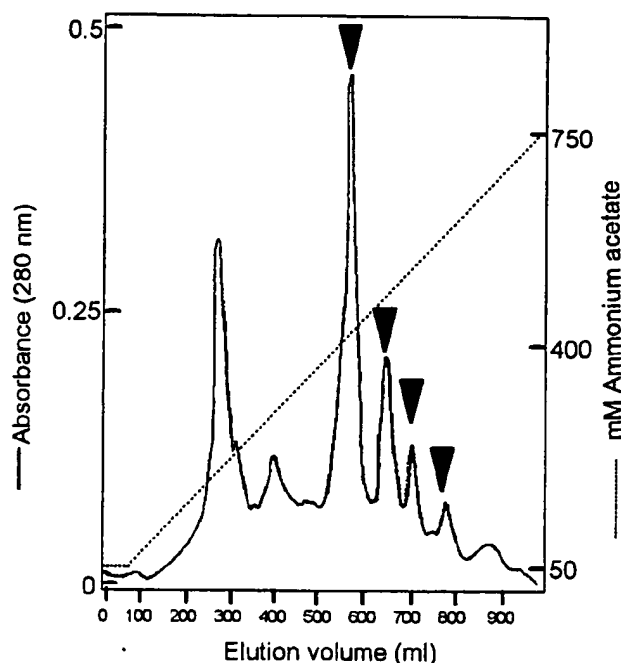


FIG. 1. Separation of proteins in the basic protein fraction from seed of *I. balsamina*. The basic protein fraction was loaded on a S-Sepharose high performance cation-exchange column in equilibrium with 50 mM NH_4Ac at pH 6. The column was washed with 50 mM NH_4Ac until the absorbance at 280 nm dropped below 0.01 absorbance unit, and the bound fraction eluted at 3 ml/min with a linear gradient of 50–750 mM NH_4Ac at pH 6 over 325 min. The eluate was monitored for proteins at 280 nm and collected in 10-ml fractions. Aliquots from each fraction were freeze-dried, resuspended in sterile water, and assayed for antifungal activity using a *F. culmorum* spore germination assay. Peaks of absorbance at 280 nm that correspond to fractions with antifungal activity when tested at 100 μ g of total protein/ml or below in the assays are arrowheaded.

28 °C in the dark, and material was harvested at 24-h intervals.

For Northern analysis, total RNA was isolated from seed as described by Jepson *et al.* (17) and blotted from a 1.5% (w/v) agarose gel onto Hybond-N membrane (Amersham). Hybridization was performed in 0.25% (w/v) Marvel (Premier Beverages, UK), 5 \times SSPE, 0.01% (w/v) SDS at 65 °C and washing in 0.2 \times SSC, 0.1% SDS at 65 °C. The [α -³²P]dCTP-labeled probe used for these studies was a purified DNA fragment representing the full length of the cDNA insert. The signal was detected by autoradiography.

For immunoblot analysis, total protein extracts were prepared from seed as described by Terras *et al.* (1). Two μ g of total protein were electroblotted from a 15% nonreducing SDS-PAGE gel onto 0.2- μ m pore size nitrocellulose membrane (Schleicher & Schuell) as described (1). The antiserum used had been raised in rabbits against bovine serum albumin-conjugated Ib-AMP1 peptide. Detection was performed using an enhanced luminescence method (Amersham) as described (1).

RESULTS

Purification and Primary Structure Determination—The basic proteins from *I. balsamina* seed were fractionated by cation exchange chromatography, and fractions assayed for antifungal activity against *F. culmorum* spores. Following chromatography, the extract yielded four peaks of activity eluting between 400 mM and 700 mM NH_4Ac (Fig. 1, arrowheaded). Fractions from each peak that showed the highest levels of activity were pooled and further purified by preparative RP-HPLC. Each pooled fraction yielded a single peak of absorbance at 210 nm which exactly matched the antifungal activity eluting from the column (data not shown). These active peaks were designated Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, respectively, according to the order of their elution from the cation exchange column.

The purified active fractions were further analyzed by SDS-

PAGE, and each was shown to contain a peptide of ~2–3 kDa (Fig. 2). The peptides ran identically whether reduced or unreduced prior to loading on the gels (data not shown), indicating that they are each composed of a single polypeptide chain.

Initial attempts to sequence the Ib-AMPs by automated Edman degradation indicated that all four peptides were N-terminally blocked. To obtain their sequences, each peptide was digested with either trypsin or chymotrypsin following modification of cysteine residues with 4-vinylpyridine, and the resulting fragments were purified by RP-HPLC prior to sequencing. The following partial amino acid sequences were generated for peptides Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4, respectively: GRRCCGWGPGRRYCVRWX, GRRCCN_xGPGRRYCKRWC, RHRCCAAGPGRKYCKRWC, and GRRCCGWGPGRRYCRRWC. Residue 7 of Ib-AMP2 could not be unambiguously identified as one of the common amino acids. Clearly, all four peptides are very close homologues of each other, with as little as one, and no more than five, amino acid differences between any pairwise alignment of the above sequences.

Electrospray Mass Spectrometry—The molecular mass of each of the purified Ib-AMP peptides was experimentally determined by electrospray mass spectrometry as 2464.6, 2527.4, 2536.6, and 2522.6 Da for Ib-AMP1, -2, -3, and -4, respectively (data not shown). These molecular mass determinations are consistent with the estimation based on SDS-PAGE.

Antifungal Activity of the Ib-AMPs—The antifungal activity of the purified peptides was assessed on 13 fungal strains, many of which are plant pathogens of significant importance to agriculture, using a standard antifungal activity assay (14). In medium A, all four peptides showed similar levels of broad spectrum activity (Table I, medium A). For the majority of the assays in this medium, the IC₅₀ values were <10 µg/ml. The antifungal activity of the peptides is, however, sensitive to the ionic strength of the assay medium and in the same medium supplemented with 1 mM CaCl₂ and 50 mM KCl, the activity of

Ib-AMP1, Ib-AMP2, and Ib-AMP3 is severely reduced (Table I, medium B). Only Ib-AMP4 maintains any significant inhibitory activity even though its activity is also markedly reduced. On some fungi, notably *F. culmorum*, the Ib-AMPs cause a very distinct swelling and hyperbranching in the spore germination assay at subinhibitory rates (Fig. 3A). The Ib-AMPs also inhibit the growth of germlings, and in the case of Ib-AMP4 this is also apparent in medium B (Table I). On germlings, the Ib-AMPs cause swelling and branching along the length of hyphae and at the hyphal tip (Fig. 3B).

Antibacterial Assays—In addition to their broad spectrum antifungal activity, the Ib-AMPs are also inhibitory to the growth of a range of bacteria, especially Gram-positive bacteria (Table II). On the Gram-positive bacteria tested, the IC₅₀ values of Ib-AMP4 are lower than those obtained with the antibiotic peptide magainin I (18).

Human Cell Integrity Assays—The effect of Ib-AMP2 and Ib-AMP4 on human erythrocytes and cell cultures of human

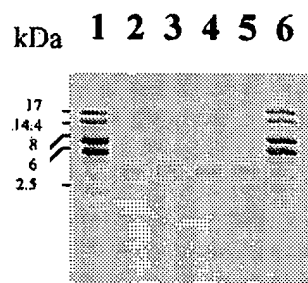


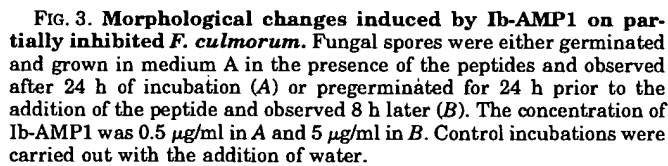
FIG. 2. SDS-PAGE analysis of the purified Ib-AMPs. 200 ng of each peptide were dissolved in reducing sample buffer and separated on a High Density Phastgel (Pharmacia). Peptides were transferred to nitrocellulose by capillary blotting and visualized by silver staining. Lanes 1 and 6, myoglobin fragments with molecular masses in kDa as indicated; lane 2, Ib-AMP1; lane 3, Ib-AMP2; lane 4, Ib-AMP3; lane 5, Ib-AMP4.

TABLE I
Antifungal activity of the Ib-AMP peptides

Protein concentrations required for 50% growth inhibition 48 h after addition were determined from dose-response curves. Proteins were added either directly to spores or to 24-h pregerminated mycelium (germlings). Assays were carried out either in medium A or in medium A supplemented with 1 mM CaCl₂ and 50 mM KCl (medium B).

Fungus	IC ₅₀			
	Ib-AMP1	Ib-AMP2	Ib-AMP3	Ib-AMP4
	µg/ml			
Medium A, spores				
<i>Alternaria longipes</i>	3	12	6	3
<i>Botrytis cinerea</i>	12	25	6	6
<i>Cladosporium sphaerospermum</i>	1	6	3	1
<i>F. culmorum</i>	1	6	6	1
<i>Penicillium digitatum</i>	3	6	3	3
<i>T. viride</i>	6	12	12	6
<i>V. alboatrum</i>	3	12	6	6
Medium B, spores				
<i>Alternaria longipes</i>	50	>200	>200	12
<i>Botrytis cinerea</i>	>200	>200	>200	200
<i>Cladosporium sphaerospermum</i>	50	>200	100	6
<i>F. culmorum</i>	50	>200	100	6
<i>Penicillium digitatum</i>	200	>200	100	25
<i>T. viride</i>	>200	>200	>200	150
<i>V. alboatrum</i>	>200	>200	>200	50
Medium B, germlings				
<i>Colletotrichum gloeosporioides</i>	ND ^a	ND	ND	25
<i>Gloeodes pomigena</i>	ND	ND	ND	>100
<i>Gloeosporium solani</i>	ND	ND	ND	>100
<i>Nectria galligena</i>	ND	ND	ND	6
<i>Phialophora malorum</i>	ND	ND	ND	6
<i>Sclerotinia sclerotiorum</i>	ND	ND	ND	25

^a ND, not determined.



cDNA Isolation and Characterization—A total of 12 cDNA clones were isolated from the dry seed cDNA library by hybrid-

Protein concentrations required for 50% growth inhibition were determined after 24 h of incubation.

Bacteria	IC ₅₀		
	Ib-AMP1	Ib-AMP4	Magainin I
	$\mu\text{g/ml}$		
Gram-positive bacteria			
<i>Bacillus subtilis</i>	10	5	20
<i>Micrococcus luteus</i>	10	5	20
<i>Staphylococcus aureus</i>	30	20	30
<i>Streptococcus faecalis</i>	6	5	20
Gram-negative bacteria			
<i>Erwinia amylovora</i>	ND ^a	>100	ND
<i>Escherichia coli</i>	>500	>500	ND
<i>Proteus vulgaris</i>	>500	>500	ND
<i>Pseudomonas solanacearum</i>	>500	>100	ND
<i>X. campestris</i>	ND	6	ND
<i>X. oryzae</i>	ND	15	ND

^a ND, not determined.

M V Q K G V V F G V L L L L L F I C S T L T S A D S K P

1 ATTTT TAGGTGAGGAAAAATGGTCCAAAAAGGTGTAGTCTTTGGGGTGCTCCTAATCTCTTCATCTGCTCTACGCTCACTTCGGCCGATTCAAGCCAA

N P T K E E E P A K K P D E V S V K S G G P E V S E D Q Y R H R C

101 ACCCTACGAAAGAGGAAGAACCAGCGAAGAAACCGGATGAGGTCAGCGTAAAGAGCGGTGGACCGGAGGTGTCGGAGGATCAATACCGTCATCGGTGCTG

A W G P G R K Y C K R W C A N A E E A A A A I P E A S E E L A Q E

201 CGCTTGGGGACCTGGGCGAAAAATATTGCAAGCGGTGGTGTGCTAACGCTGAAGAGGCGGGCGCCGAATCCCCGAGGCAAGTGAAGAATTAGCTCAGGAG

E A P V Y S E D Q W G R R C C G W G P G R R Y C V R W C Q N A E E

301 GAGGCTCCGGTGTACTCGGAGGATCAGTGGGGTCTGTCGGTGTCTGCGGCTGGGGACCCGGCCGAAGATACTGCGTCCGCTGGTGTCAAACCGCGAAGAGG

=====IbAMP1-C===== <=====IbAMP1-B=====

A A A A I P E A T E K A Q E A P V Y S E D Q W G R R C C G W G P G

401 CGGCCGCGCAATCCCCGAGGCGACTGAAAAGCTCAGGAGGCTCCGGTGACTCGGAGGATCAGTGGGGTCTCGATGCTGCGGCTGGGACCCGCGCC

R Y C V R W C Q N A E E A A A A V A I P E A S E K A Q E G P V Y S

501 ACGGTATTGCTGCGCTGGTGTCAAACCGCGAAGAGGCGCGCGCGGTGGCAATCCCCGAGGCAAGTGAAGAACTCAGGAGGGACCCGTGTACTCG

E D Q W G R R C C G W G P G R R Y C V R W C S N A A D E V A T P E

601 GAGGATCAGTGGGGTCCCGATGCTGCGGTTGGGGACCTGGCCGTAGGTATTGCTGCGGTGGTGCAGCAACGCCGCCGACGAGGTGGCAACACCCGAGG

D V E P G Q Y G R R C C N G P V G R R Y C K R W C H N A A E E A T L

701 ACGTAGAACCGGGTCAGTACGGTCTGCGTGTGCAACTGGGACCTGGGCGAAGGTATTGCAAGCGGTGGTGTCTATAATGCGGCTGAAGAGGCAACTCT

K A F E E E A A R E Q P V Y S E D Q W G R R C C G W G P G R R Y C

801 CAAGGCATTGAAGAGGAAGCAGCTCGGGAGCAACCGGTGTACTCGGAGGACAGTGGGGTCCCGGTGCTGCGGTGGGGACCCGGCGGTAGGTACTGC

R R W C Q S A E E A A A F Q A G E V T A S L M L I M F K A C P C M

901 AGCGCGTGGTGTCAAAGCGCCGAAGAAGCGGCTGCGTTCAGGCTGGGAGGTAAGTGTCTCTTGATGCTCATCATGTTTAAGGATGCCCATGTCATGG

G P V P S V *

1001 GGCCGGTGCCTTCTGTTTAAGGCCACTCTAGCTAGCTACGTACTCTTAATAAGGGCACATGAAAAGTTTGTCCTTTAGAAATAAGGCACAGTAAGAAAT

1101 AAAATGTCCAACCTTCTTTTATGAAGAAGTGACAAATAAGTGAAGCTGAATAATATATATTGTGACACGTTTGTTGTTGTACAAAAATAACATCTTTTC

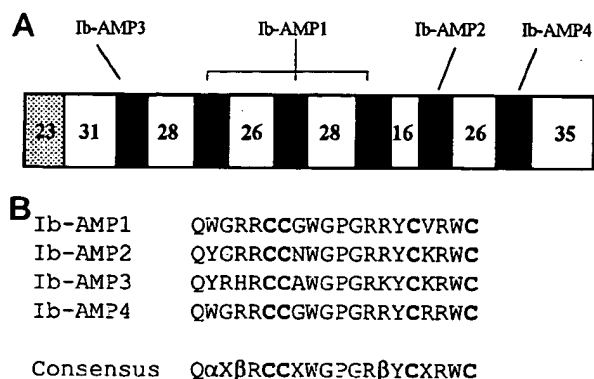
1201 AGATGAACAACCTTTAATGGAAGAAAAAAGAAAAA (5)

~~~~~AACTTTTATTAGTTATTA (A<sub>n</sub>) (3)

~~~~~AACTTTTATTAGTTATTACCTA (A<sub>n</sub>) (2)

~~~~~AACTTTTATTAGTTATTACCTAGA (A<sub>n</sub>) (1)

FIG. 4. Complete nucleotide sequence of cDNA clone Ib22 insert and the predicted translation product. Amino acid residues comprising the domains representing the mature Ib-AMP peptides are in **bold** and **boxed**; those comprising the predicted signal sequence are underlined. The predicted termination codon is *double underlined*. The DNA sequences immediately upstream of poly(A) tails in otherwise identical cDNA clones are shown. The number of independently isolated cDNA clones exhibiting these different sites of polyadenylation is indicated in *parentheses*. The annealing positions of the named degenerate oligonucleotide PCR primers used to generate the initial hybridization probe are indicated by *double arrows* underneath the first of the Ib-AMP1 repeat regions.

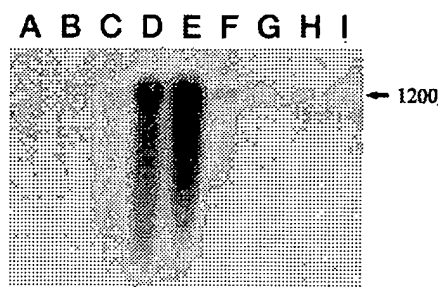


**FIG. 5. General gene structure and alignment of peptide sequences.** A, the generalized structure of the predicted 333 amino acid primary translation product in which the individual 20-amino acid domains representing the mature Ib-AMP peptides are shaded, with the Ib-AMP member indicated immediately above. The prepeptide regions are unshaded, the predicted prepeptide (signal sequence) region is hatched, and the number of amino acids comprising each of these regions is indicated. B, amino acid sequence alignment of the predicted translation products of each mature domain. A consensus sequence for the Ib-AMPs appears below in which upper case letters represent invariant residues, α an aromatic residue, β a basic residue, and X a nonconserved residue. Cysteine residues are in bold text.

ization, and the insert of the largest clone (Ib22) fully sequenced following *in vivo* excision of the phagemid. The 1238-base pair DNA sequence of the insert from this clone is presented in Fig. 4 and discussed further below. Approximately 500 base pairs of DNA sequence were obtained for each of the other cDNA clones. Although some clones appeared to be truncated at the 5' end, the DNA sequence of each was identical to that of clone Ib22 in regions of overlaps, the only differences being apparently due to the exact position of polyadenylation. As indicated in Fig. 4, there appeared to be 5 different polyadenylation sites utilized among the 12 cDNA clones within a region spanning 52 base pairs of the 3'-untranslated region, the majority being at the same position as that of clone Ib22.

Clone Ib22 is considerably larger than would be anticipated for a cDNA encoding a single IbAMP peptide. In fact it contains an open reading frame encoding a predicted protein comprising 333 amino acids, with a molecular mass of 37,262 Da. Analysis of this predicted translation product reveals within it the presence of domains that correspond exactly in amino acid sequence to the four antimicrobial peptides described above. Ib-AMP1 is represented three times in consecutive repeats, the other Ib-AMPs once each. The six boxed regions in Fig. 4 represent, consecutively, Ib-AMP3, Ib-AMP1, Ib-AMP1, Ib-AMP1, Ib-AMP2, and Ib-AMP4. Hydropathy plots and sequence analysis (not shown) of the predicted translation product deriving from clone Ib22 predict a 23-amino acid N-terminal signal sequence, consistent with the fact that many plant antifungal peptides are extracellularly located (1). The general structure of the predicted translation product and an amino acid alignment of those regions assumed to encode the individual mature peptides are presented in Fig. 5.

The proprotein domains flanking each Ib-AMP peptide domain vary in length between 16 and 35 amino acids but display some degree of homology with each other, each containing at least five negatively charged amino acids, generally organized as doublets in the vicinity of the presumed cleavage sites (see Fig. 4). This composition is in contrast to the highly basic Ib-AMP domains, which are separated by these proprotein regions. Although there is no clear homology to other peptide sequences or processing sites in the data bases, it is assumed that these regions contain information required for the correct



**FIG. 6. A Northern blot of total RNA from developing, mature, and germinating *I. balsamina* seed.** Total RNA isolated from developing seed was from the developmental stages described under "Experimental Procedures." Each lane on the 1.5% (w/v) agarose gel was loaded with 5 µg of total RNA. Northern blots were probed with radiolabeled fragment representing the insert of cDNA clone Ib22. The arrow indicates the length, in nucleotides, of the hybridizing band as determined by comparison with the migration of RNAs of known lengths (not shown). Sources of extracts on the gel were as follows: lanes A-D, seed at developmental stages 2-5, respectively; lane E, dry mature seed; lanes F-I, seed at 24, 48, 72, and 96 h postgermination, respectively.

processing of the preproprotein into constituent mature peptides.

**Analysis of Expression**—To investigate the accumulation of Ib-AMP-related transcripts and protein during seed development, both Northern and Western blot analysis was performed on material isolated from developing, dry, and germinating seed. The hybridization pattern resulting from Northern blots probed with the entire insert of clone Ib22 is shown in Fig. 6. There appears to be hybridization to a single class of transcript of ~1200 nucleotides, which is in accordance with the size predicted from the cDNA sequence analysis of clone Ib22. The pattern of hybridization indicates that the highest accumulation of related transcript is found in dry seed (Fig. 6, lane E) and in the stage of development immediately prior to this (Fig. 6, lane D).

Western blots using Ib-AMP1 antibody indicate that immunoreactive material is most abundant in dry seed, from which the Ib-AMPs were originally isolated, and in seed undergoing the early stages of germination (Fig. 7, lanes E, F, and G). There appear to be significant quantities of such material also present in the developmental stage immediately preceding seed dry-down (Fig. 7, lane D). Immunoreactive material migrating at a higher position on the gel (Fig. 7, lane C) may represent unprocessed or incompletely processed precursor protein. An investigation into the processes involved in maturation of the precursor protein is currently under way.

#### DISCUSSION

Four closely related, small, basic, cysteine-rich peptides have been purified from seed of *I. balsamina* and shown to be active *in vitro* against a range of fungal and bacterial species. The majority of the amino acid sequence of the four peptides could be determined experimentally, although complete assignment was prevented because the N terminus of each was blocked. The molecular mass of Ib-AMP1 as determined by electrospray mass spectrometry indicated that the full-length Ib-AMP1 sequence was only 2 amino acids longer at its N terminus than the 18 amino acids assigned by direct amino acid sequencing. It can be predicted that the amino acid residue N-terminally adjacent to the sequenced region must be either a tryptophan or a tyrosine for chymotrypsin to have cleaved Ib-AMP1 in that position. Furthermore, based on the molecular mass estimation and the fact that the peptide is N-terminally blocked, it can be considered likely that the unassigned N-terminal residue is a cyclized glutamine. Both these speculations were indeed confirmed for all four Ib-AMP peptides by the subsequent analysis

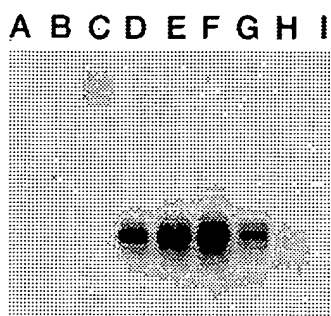


FIG. 7. An immunoblot of protein extracts from developing, mature, and germinating *I. balsamina* seed. Total protein was isolated from seed at the developmental stages described under "Experimental Procedures." Each lane on the 15% (w/v) nonreducing SDS-PAGE gel was loaded with 2  $\mu$ g of total protein extract. Western blots were immunostained with an antiserum raised against Ib-AMP1. Sources of extracts on the gel were as follows: lanes A–D, seed at developmental stages 2–5, respectively; lane E, dry mature seed; lanes F–I, seed at 24, 48, 72, and 96 h postgermination, respectively.

of the cDNA clone reported here.

The molecular mass of each peptide determined experimentally by matrix-assisted laser desorption/ionization-time of flight electrospray analysis is consistently ~21 Da lower than that theoretically predicted for each by direct translation of the gene sequence. This difference is precisely accounted for if it is assumed that the N-terminal glutamine residue of each Ib-AMP peptide is cyclized and that the four internal cysteine residues form two disulfide bridges. The former supposition is supported by the fact that all classes of the Ib-AMP peptides were found to be N-terminally blocked when amino acid sequencing was attempted. The latter is supported by the fact that the native Ib-AMP peptides do not react with Ellman's free thiol reagent.

When the theoretical molecular masses for Ib-AMP1, Ib-AMP2, Ib-AMP3, and Ib-AMP4 are adjusted to take into account the N-terminal cyclization and disulfide bridge formation, the respective values of 2464.3, 2527.4, 2536.5, and 2521.4 match the experimentally determined values of 2464.6, 2527.4, 2536.6, and 2522.6 Da almost exactly. This provides further evidence that the gene represented by the Ib22 cDNA does indeed encode all four classes of isolated peptides. Furthermore, these data confirm that the unassigned residue of Ib-AMP2 is a tryptophan, as predicted from the cDNA sequence. Interestingly, a residue of the plant defensin peptide *Br*-AFP2 could not be identified by direct sequencing (11) but might reasonably be assumed to be a tryptophan residue because of close homology to other Brassicaceae antifungal proteins.

The Ib-AMP family is clearly distinct from all other families of plant antimicrobial peptides reported previously (Ref. 19; Table III). Searches of protein data bases did not identify any peptides or proteins with significant homology to either the mature Ib-AMP peptides or the intervening propeptide regions. Limited homology was noted between the Ib-AMPs and the N-terminal region of the mature peptide domain of the eight-cysteine type plant thionins, particularly with respect to the cysteine residue pattern (Table III). This might indicate an evolutionary relationship between the Ib-AMP and thionin genes involving processes of truncation and duplication. In addition, the sequence Gly-Pro-Gly-Arg-Arg-Tyr, corresponding to the conserved residues 10–15 in Ib-AMP1, Ib-AMP2, and Ib-AMP4, was identified in a number of other proteins, notably viral proteins (22). In a number of these proteins, this sequence has been shown to form a  $\beta$ -turn, and it is possible that a similar turn is occurring at this sequence in Ib-AMP1.

All four Ib-AMPs are inhibitory to the growth of a wide range

of filamentous fungi when assayed in medium A. When this medium is supplemented with  $\text{CaCl}_2$  and KCl, only Ib-AMP4 retains any significant inhibitory activity, even though it differs from the more sensitive Ib-AMP1 by only a single amino acid residue substitution. A reduction in antifungal activity in media of increased ionic strength is a common feature of most of the small cationic peptides isolated to date and probably reflects the weakening of electrostatic interactions with the target rather than any alteration of the structure of the peptide by the binding of ions from the medium (11). This is supported by the fact that the degree of reduction in antifungal activity is dependent on the test fungus (Table I). Moreover, the finding that the only one of the four Ib-AMP peptides that remains active in medium B is the most basic homologue would also support this. On the whole, the antifungal activity of the Ib-AMPs compares favorably with the more active antifungal and antimicrobial peptides purified to date from plants (2–4, 8, 10, 11).

In addition to their antifungal activity, the Ib-AMPs were also inhibitory to the growth of the four Gram-positive bacteria that were tested and to the growth of two Gram-negative *Xanthomonas* species. In these assays, their activity was compared with the antibiotic peptide magainin I (18), and on all four of the Gram-positive bacteria tested the Ib-AMPs were equally active as or more active than magainin I. Few of the other antifungal peptides isolated to date from plants show significant levels of activity on bacteria (2, 14).

From the literature, it is apparent that peptides with very different functions can share a common structure. The  $\alpha$ -conotoxins, which have been purified from marine snails, have a similar cysteine arrangement to the Ib-AMPs, although the spacing between the two C-terminal cysteines is different (23). The solution structure for  $\alpha$ -conotoxin GI has been determined using two-dimensional NMR and shows that the two disulfide bonds stabilize a nonrandom coil structure with two  $\beta$ -turns (24). Preliminary work involving the analysis of purified products released from unreduced Ib-AMP1 following digestion by trypsin showed that Cys<sub>16</sub> could not be connected to Cys<sub>20</sub> but rather that the C-terminal cysteine was connected to either Cys<sub>6</sub> or Cys<sub>7</sub> (data not shown). The two remaining possible combinations of pairwise connectivities (*i.e.* Cys<sub>6</sub>-Cys<sub>16</sub> and Cys<sub>7</sub>-Cys<sub>20</sub> or Cys<sub>6</sub>-Cys<sub>20</sub> and Cys<sub>7</sub>-Cys<sub>16</sub>) could not be resolved by protease digestion methods. However, NMR has since been used to determine a solution structure for Ib-AMP1, enabling a comparison with that of the  $\alpha$ -conotoxins, and will be reported elsewhere.<sup>2</sup>

The amino acid sequence of each of the four Ib-AMP peptides isolated from dry seed can be identified within the predicted translation product of a single class of cDNA obtained from RNA also isolated from dry seed. One of the peptides is represented three times, the other three once. The fact that 12 individual cDNAs have essentially identical DNA sequences suggests that, at least in seed, only a single gene encoding such peptides is expressed. Southern blotting of genomic DNA suggests that there is only one gene (data not shown).

cDNAs encoding antibacterial peptides processed from a multi-peptide precursor have been described previously, albeit not in plants. Inducible, proline-rich, 18-amino acid apidaecin peptides from bees are encoded by a family of cDNAs which contain up to 12 peptide repeats separated by well conserved "processing" regions (25). The cDNA sequence coding for *Xenopus* prepro-magainins also has a multi-peptide structure (26). In plants, there are two published examples of cDNAs encoding multi-peptide precursors. The first well known example is that

<sup>2</sup> S. Patel and J. Thornton, personal communication.

TABLE III  
Comparison of plant antifungal peptides

The size of the mature peptide (number of amino acids) and spacing of cysteine residues within its sequence is presented for Ib-AMP1 and representative members of other reported plant antifungal peptide families. Figures in the final column represent the number of amino acid residues flanking the cysteine residues indicated.

| Peptide family         | Representative member | Ref.       | Size | Spacing of cysteine residues       |
|------------------------|-----------------------|------------|------|------------------------------------|
| Plant defensins        | Rs-AFP2               | 1, 10      | 51   | 3-C-10-C-5-C-3-C-9-C-8-C-1-C-3-C   |
| Knottin-type           | Mj-AMP1               | 2          | 36   | 1-C-6-C-8-CC-3-C-10-C-3            |
| Lipid transfer protein | Ac-AMP1               | 8          | 93   | 3-C-9-C-12-CC-18-C-1-C-23-C-15-C-4 |
| Hevein-type            | Ac-AMP2               | 3          | 30   | 3-C-4-C-4-CC-5-C-6-C-2             |
| Macadamia              | Mi-AMP1               | 20         | 76   | 10-C-9-C-1-C-25-C-14-C-11-C        |
| Maize basic protein    | MBP-1                 | 4          | 33   | 6-C-3-C-13-C-3-C-4                 |
| Thionin (8-Cys type)   | $\alpha$ -Purothionin | 21         | 45   | 2-CC-7-C-3-C-8-C-3-C-1-C-8-C-6     |
| Impatiens              | Ib-AMP1               | This paper | 20   | 5-CC-8-C-3-C                       |

of polyubiquitin cDNA, which encodes head to tail repeats of ubiquitin peptides, apparently without linker domains (27). The other example is that of a cDNA from *Nicotiana glauca* encoding stigma proteinase inhibitor peptides separated from each other by dibasic dipeptide linkers (28). No structure similar to that of the Ib-AMP precursor, with acidic linker domains separating basic mature peptide domains, has yet been reported in plants. The mechanism of processing of the Ib-AMP preproprotein into its constituent mature peptides is not apparent from the inferred amino acid sequence of the translation product of the cDNA since no previously well characterized processing sites are apparent within the preproprotein on the basis of sequence homology alone. However, the presence of negatively charged amino acid doublets (EE or DE) at equivalent positions within each propeptide (including immediately upstream of five of the six mature peptide regions) might suggest the presence of specific processing sites.

The above examples of multiple mature peptides being generated from a single precursor differ in many respects from each other and from the Ib-AMP gene structure described here, possibly reflecting differences in the mechanism of processing. Nevertheless, it is reasonable to assume that concatenation of such small peptide domains is a general mechanism for enhanced simultaneous production of related peptides. Should these related peptides individually exhibit different activity profiles, such a mechanism would enable a broader activity spectrum to be achieved from a single gene transcript.

The mode of action of the Ib-AMPs is presently unknown. Of particular interest is whether the peptides are interacting directly with microbial membranes or whether they have a protein/receptor target. Even at very high rates (500  $\mu$ g/ml), the Ib-AMPs do not cause any visible cell lysis or membrane collapse on fungi. The peptides were shown not to affect human cells, and they are noncytotoxic to cultured insect and plant cells (data not shown). Taken together, these preliminary data suggest that the Ib-AMPs are not acting as ionophores but rather that they are inhibiting a distinct cellular process.

As a rough estimate from the yields obtained, the Ib-AMPs account for ~0.5% of the total protein in mature *I. balsamina* seed, where they may play a role in the defense of the germinating seed. Assuming that genes in dry seed are generally transcriptionally inactive, the patterns of hybridizing mRNA revealed by Northern blot hybridization analysis might indicate that the gene is highly transcribed immediately prior to seed maturation and that the RNA remains undegraded in dry seed. Within 24 h of imbibition, the RNA level is markedly decreased, whereas protein levels are at a peak, perhaps suggesting that RNA stored in dry seed is translated and rapidly degraded at the onset of germination. It is not known whether the expression of the Ib-AMP peptides is limited to the seed, but as in the case of other plant antimicrobial peptides (1, 29) the Ib-AMPs may also be present, perhaps inducibly, in other

tissues of the plant and may play a more general role in protecting the plant from microbial infections.

**Acknowledgments**—We thank S. Aitken for the preparation of some later samples of Ib-AMP peptides and L. Hunt (Protein Sequencing Unit, University of Southampton) for amino acid sequence analysis. The mass spectrometry data were provided by M-Scan Ltd., Ascot, UK. The authors also acknowledge J. Manners (University of Queensland, Australia), T. Sutton (North Carolina State University), D. Sugar (Oregon State University), E. De Bruyne (Société Européenne de Semences, Tienen, Belgium), E. Chevreau (Institut National de la Recherche Agronomique, Angers, France), S. Seal (National Resource Institute, Kent, UK), and G. Spangenberg (Eidgenössische Technische Hochschule (ETH), Zurich, Switzerland) for kindly providing fungal or bacterial strains.

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Search Results - Record(s) 1 through 45 of 45 returned.

☐ 1. Document ID: US 20030096985 A1

Using default format because multiple data bases are involved.

L6: Entry 1 of 45

File: PGPB

May 22, 2003

PGPUB-DOCUMENT-NUMBER: 20030096985

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030096985 A1

TITLE: Small and cysteine rich antifungal defensin and thionin-like protein genes highly expressed in the incompatible interaction

PUBLICATION-DATE: May 22, 2003

## INVENTOR-INFORMATION:

| NAME            | CITY    | STATE | COUNTRY | RULE-47 |
|-----------------|---------|-------|---------|---------|
| Oh, Bounng-Jun  | Kwangju |       | KR      |         |
| Kyung Ko, Moon  | Kwangju |       | KR      |         |
| Shin, Byongchul | Kwangju |       | KR      |         |

US-CL-CURRENT: 536/23.6; 435/320.1, 536/23.1

|      |       |          |       |        |                |      |           |           |             |        |      |          |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|

☐ 2. Document ID: US 6770750 B2

L6: Entry 2 of 45

File: USPT

Aug 3, 2004

DOCUMENT-IDENTIFIER: US 6770750 B2.

TITLE: Small and cysteine rich antifungal defensin and thionin-like protein genes highly expressed in the incompatible interaction

|      |       |          |       |        |                |      |           |           |             |        |      |          |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|

☐ 3. Document ID: US 6187995 B1

L6: Entry 3 of 45

File: USPT

Feb 13, 2001



DOCUMENT-IDENTIFIER: US 6187995 B1

TITLE: Method for producing disease resistant plant with thionin gene from Avena sativa

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Drawings |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|

☐ 4. Document ID: US 5990389 A

L6: Entry 4 of 45

File: USPT

Nov 23, 1999

DOCUMENT-IDENTIFIER: US 5990389 A

TITLE: High lysine derivatives of .alpha.-hordothionin

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Drawings |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|

☐ 5. Document ID: US 5885802 A

L6: Entry 5 of 45

File: USPT

Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885802 A

TITLE: High methionine derivatives of .alpha.-hordothionin

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Drawings |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|

☐ 6. Document ID: US 5885801 A

L6: Entry 6 of 45

File: USPT

Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885801 A

TITLE: High threonine derivatives of .alpha.-hordothionin

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Drawings |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|----------|

☐ 7. Document ID: US 5703049 A

L6: Entry 7 of 45

File: USPT

Dec 30, 1997

DOCUMENT-IDENTIFIER: US 5703049 A

TITLE: High methionine derivatives of .alpha.-hordothionin for pathogen-control

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstracts | Abstracts | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|
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☐ 8. Document ID: US 5168066 A

L6: Entry 8 of 45

File: USPT

Dec 1, 1992

DOCUMENT-IDENTIFIER: US 5168066 A

TITLE: Thionin staining and imaging technique

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstracts | Abstracts | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|
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☐ 9. Document ID: US 3299035 A

L6: Entry 9 of 45

File: USPT

Jan 17, 1967

DOCUMENT-IDENTIFIER: US 3299035 A

TITLE: L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucyl-glycyl-L-leucyl-L-methionin-amide and its acid addition salts [TEXT AVAILABLE IN USOCR DATABASE]

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstracts | Abstracts | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|

☐ 10. Document ID: US 2794786 A

L6: Entry 10 of 45

File: USPT

Jun 4, 1957

DOCUMENT-IDENTIFIER: US 2794786 A

TITLE: Thionin dye-ion exchange resin indicator compounds [TEXT AVAILABLE IN USOCR DATABASE]

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstracts | Abstracts | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-----------|--------|------|----------|

☐ 11. Document ID: US 2014519 A

L6: Entry 11 of 45

File: USPT

Sep 17, 1935

DOCUMENT-IDENTIFIER: US 2014519 A

TITLE: Argento-chrome tetramethylthionin and process of manufacture [TEXT AVAILABLE

IN USOCR DATABASE]

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Abstract | Claims | KMC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|-----|----------|
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☐ 12. Document ID: WO 132849 A2

L6: Entry 12 of 45

File: EPAB

May 10, 2001

PUB-NO: WO000132849A2

DOCUMENT-IDENTIFIER: WO 132849 A2

TITLE: INHIBITORS OF VEGETABLE CYSTATHIONIN GAMMA SYNTHASE

PUBN-DATE: May 10, 2001

## INVENTOR-INFORMATION:

| NAME                    | COUNTRY |
|-------------------------|---------|
| LABER, BERND            | DE      |
| CLAUSEN, TIM            | DE      |
| HUBER, ROBERT           | DE      |
| STEEGBORN, CLEMENS      | DE      |
| MESSERSCHMIDT, ALBRECHT | DE      |

INT-CL (IPC): C12 N 9/88; C30 B 29/58; C12 Q 1/527; A01 N 57/24; A01 N 43/76EUR-CL (EPC): A01N043/82; A01N057/24, C12N009/88

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Abstract | Claims | KMC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|-----|----------|
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☐ 13. Document ID: WO 9950390 A1

L6: Entry 13 of 45

File: EPAB

Oct 7, 1999

PUB-NO: WO009950390A1

DOCUMENT-IDENTIFIER: WO 9950390 A1

TITLE: METHIONIN CONTAINING ANIMAL CELL CULTURE MEDIUM AND ITS USE

PUBN-DATE: October 7, 1999

## INVENTOR-INFORMATION:

| NAME            | COUNTRY |
|-----------------|---------|
| JAREKRANS, MATS | SE      |
| OLOVSSON, HANS  | SE      |

INT-CL (IPC): C12 N 5/00; C07 K 14/555EUR-CL (EPC): C12N005/00; C07K014/56

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Abstract | Claims | KMC | Draw. De |
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☐ 14. Document ID: EP 902089 A2

L6: Entry 14 of 45

File: EPAB

Mar 17, 1999

PUB-NO: EP000902089A2

DOCUMENT-IDENTIFIER: EP 902089 A2

TITLE: Disease resistant plant including thionin gene

PUBN-DATE: March 17, 1999

## INVENTOR-INFORMATION:

| NAME                | COUNTRY |
|---------------------|---------|
| OHASHI, YUKO        | JP      |
| MITSUHARA, ICHIRO   | JP      |
| OHSHIMA, MASAHIRO   | JP      |
| UGAKI, MASASHI      | JP      |
| HIROCHIKA, HIROHIKO | JP      |
| HONKURA, RYOSO      | JP      |
| IWAI, TAKAYOSHI     | JP      |
| NAKAMURA, SHIGEO    | JP      |

INT-CL (IPC): C12 N 15/82; C12 N 15/29; A01 H 5/00; A01 N 65/00

EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
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☐ 15. Document ID: WO 9641608 A2

L6: Entry 15 of 45

File: EPAB

Dec 27, 1996

PUB-NO: WO009641608A2

DOCUMENT-IDENTIFIER: WO 9641608 A2

TITLE: PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES

PUBN-DATE: December 27, 1996

## INVENTOR-INFORMATION:

| NAME              | COUNTRY |
|-------------------|---------|
| VERNON, LEO P     |         |
| RAEL, EPIE D      |         |
| GASANOV, SARDAR E |         |

INT-CL (IPC): A61 K 0/

EUR-CL (EPC): A61K035/78; C07K016/28, A61K047/48 , A61K047/48

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
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☐ 16. Document ID: WO 9638563 A1

L6: Entry 16 of 45

File: EPAB

Dec 5, 1996

PUB-NO: WO009638563A1  
DOCUMENT-IDENTIFIER: WO 9638563 A1  
TITLE: HIGH METHIONINE DERIVATIVES OF alpha -HORDOTHIONIN

PUBN-DATE: December 5, 1996

## INVENTOR-INFORMATION:

NAME

COUNTRY

RAO, GURURAJ A

INT-CL (IPC): C12 N 15/29; C12 N 15/82; C07 K 14/415; C12 N 5/10; A01 H 5/00  
EUR-CL (EPC): C07K014/415; C12N015/82, C12N015/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Draw. Data |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|------------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|------------|

☐ 17. Document ID: WO 9638562 A1

L6: Entry 17 of 45

File: EPAB

Dec 5, 1996

PUB-NO: WO009638562A1  
DOCUMENT-IDENTIFIER: WO 9638562 A1  
TITLE: HIGH THREONINE DERIVATIVES OF alpha -HORDOTHIONIN

PUBN-DATE: December 5, 1996

## INVENTOR-INFORMATION:

NAME

COUNTRY

RAO, ARAGULA GURURAJ

US

INT-CL (IPC): C12 N 15/29; C12 N 15/82; C07 K 14/415; A01 H 5/00  
EUR-CL (EPC): C07K014/415; C12N015/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWC | Draw. Data |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|-----|------------|
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☐ 18. Document ID: WO 9416078 A2

L6: Entry 18 of 45

File: EPAB

Jul 21, 1994

PUB-NO: WO009416078A2  
DOCUMENT-IDENTIFIER: WO 9416078 A2  
TITLE: HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN

PUBN-DATE: July 21, 1994

## INVENTOR-INFORMATION:

NAME

COUNTRY

RAO, A GURURAJ

BEACH, LARRY R

INT-CL (IPC): C12N 15/29; C07K 13/00; C12N 5/10; A01H 5/00; A01N 65/00; C12N 1/21  
EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|

☐ 19. Document ID: WO 9416078 A1

L6: Entry 19 of 45

File: EPAB

Jul 21, 1994

PUB-NO: WO009416078A1

DOCUMENT-IDENTIFIER: WO 9416078 A1

TITLE: HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN

PUBN-DATE: July 21, 1994

## INVENTOR-INFORMATION:

NAME

COUNTRY

RAO, A GURURAJ

BEACH, LARRY R

INT-CL (IPC): C12N 15/29; C07K 13/00; C12N 5/10; A01H 5/00; A01N 65/00; C12N 1/21

EUR-CL (EPC): A01N065/00; C07K014/415, C12N015/82 , C12N015/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|

☐ 20. Document ID: EP 538193 A2

L6: Entry 20 of 45

File: EPAB

Apr 21, 1993

PUB-NO: EP000538193A2

DOCUMENT-IDENTIFIER: EP 538193 A2

TITLE: Condensed cycloaliphatic-amidino-hydrazone salts as S-adenosylmethionin decarboxylase inhibitors.

PUBN-DATE: April 21, 1993

## INVENTOR-INFORMATION:

NAME

COUNTRY

STANEK, JAROSLAV DR

CH

FREI, JOERG DR

CH

CARAVATTI, GIORGIO DR

CH

US-CL-CURRENT: 564/227

INT-CL (IPC): A61K 31/155; C07C 271/18; C07C 281/18

EUR-CL (EPC): C07C281/18

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|

☐ 21. Document ID: EP 445434 A2

L6: Entry 21 of 45

File: EPAB

Sep 11, 1991

PUB-NO: EP000445434A2  
DOCUMENT-IDENTIFIER: EP 445434 A2  
TITLE: Thionin stain technique.

PUBN-DATE: September 11, 1991

## INVENTOR-INFORMATION:

| NAME              | COUNTRY |
|-------------------|---------|
| ZAHNISER, DAVID J | US      |
| ODU, PETRUS S     | US      |

US-CL-CURRENT: 435/34  
INT-CL (IPC): G01N 1/30; G01N 21/84  
EUR-CL (EPC): G01N001/30

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|

☐ 22. Document ID: CN 1524881 A

L6: Entry 22 of 45

File: DWPI

Sep 1, 2004

DERWENT-ACC-NO: 2004-805494  
DERWENT-WEEK: 200480  
COPYRIGHT 2005 DERWENT INFORMATION LTD  
TITLE: Nanometer metallothionin and preparation process

INVENTOR: CUI, J; TAO, M

PRIORITY-DATA: 2003CN-0115579 (February 28, 2003)

## PATENT-FAMILY:

| PUB-NO              | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC    |
|---------------------|-------------------|----------|-------|-------------|
| <u>CN 1524881 A</u> | September 1, 2004 |          | 000   | C07K014/825 |

INT-CL (IPC): C07 K 14/825

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|

☐ 23. Document ID: US 6653463 B1

L6: Entry 23 of 45

File: DWPI

Nov 25, 2003

DERWENT-ACC-NO: 2004-050525  
DERWENT-WEEK: 200408  
COPYRIGHT 2005 DERWENT INFORMATION LTD  
TITLE: New nucleic acid molecule encoding a thionin-like protein with insecticidal and fungicidal activity, useful for providing pest and pathogen resistance to plants

INVENTOR: CHEN, C; CHEN, K ; KUAN, C ; LIN, C

PRIORITY-DATA: 2000US-0686332 (October 11, 2000)

## PATENT-FAMILY:

| PUB-NO               | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|----------------------|-------------------|----------|-------|------------|
| <u>US 6653463 B1</u> | November 25, 2003 |          | 011   | C12N015/29 |

INT-CL (IPC): C12 N 15/11; C12 N 15/29; C12 N 15/63; C12 P 21/02

| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | FIGS | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
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☐ 24. Document ID: AU 2002250729 A1, WO 200288359 A1

L6: Entry 24 of 45

File: DWPI

Nov 11, 2002

DERWENT-ACC-NO: 2003-201227

DERWENT-WEEK: 200433

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: New ryegrass or fescue nucleic acid encoding a thionin, thaumatin-like, elicitor-responsive or defensin polypeptide, for modifying disease and/or plant resistance, plant defence response and/or protein storage in a plant

INVENTOR: EMMERLING, M; ONG, E K ; SAWBRIDGE, T ; SPANGENBERG, G ; SAWBRIDGE, T I

PRIORITY-DATA: 2001AU-0004735 (May 2, 2001)

## PATENT-FAMILY:

| PUB-NO                  | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|-------------------------|-------------------|----------|-------|------------|
| <u>AU 2002250729 A1</u> | November 11, 2002 |          | 000   | C12N015/29 |
| <u>WO 200288359 A1</u>  | November 7, 2002  | E        | 195   | C12N015/29 |

INT-CL (IPC): A01 H 5/00; C07 K 14/415; C07 K 14/43; C12 N 15/29

| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | FIGS | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|

☐ 25. Document ID: US 20020150967 A1, US 6593102 B2

L6: Entry 25 of 45

File: DWPI

Oct 17, 2002

DERWENT-ACC-NO: 2003-288070

DERWENT-WEEK: 200364

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: Cytological staining solution useful in cytological or histological analysis, e.g. for imaging cells, comprises methanol, a phenol derivative and thionin

INVENTOR: ISENSTEIN, L M; LAPEN, D C ; MUI, K K ; SOULE, N W ; ZAHNISER, D J

PRIORITY-DATA: 2001US-0054578 (November 12, 2001), 1999US-0430116 (October 29, 1999)

## PATENT-FAMILY:

| PUB-NO                   | PUB-DATE         | LANGUAGE | PAGES | MAIN-IPC   |
|--------------------------|------------------|----------|-------|------------|
| <u>US 20020150967 A1</u> | October 17, 2002 |          | 027   | G01N033/48 |



US 6593102 B2

July 15, 2003

000

C12Q001/00

INT-CL (IPC): C12 Q 1/00; G01 N 1/30; G01 N 33/48

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|

☐ 26. Document ID: JP 2002272292 A

L6: Entry 26 of 45

File: DWPI

Sep 24, 2002

DERWENT-ACC-NO: 2002-718704

DERWENT-WEEK: 200278

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: A disease-resistant plant in which wasabi gamma-thionin gene is introduced, creation of the disease-resistant plant

PRIORITY-DATA: 2001JP-0083526 (March 22, 2001)

## PATENT-FAMILY:

| PUB-NO                 | PUB-DATE           | LANGUAGE | PAGES | MAIN-IPC   |
|------------------------|--------------------|----------|-------|------------|
| <u>JP 2002272292 A</u> | September 24, 2002 |          | 011   | A01H005/00 |

INT-CL (IPC): A01 H 5/00; C07 K 14/415; C12 N 15/09

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|----------|

☐ 27. Document ID: NZ 524962 A, WO 200222159 A1, AU 200075771 A, EP 1322323 A1, JP 2004508412 W

L6: Entry 27 of 45

File: DWPI

Dec 24, 2004

DERWENT-ACC-NO: 2002-415978

DERWENT-WEEK: 200506

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: Use of thionin for e.g. stimulating immunity for the treatment of tumor development

INVENTOR: VERNON, L P

PRIORITY-DATA: 2000WO-US24947 (September 11, 2000)

## PATENT-FAMILY:

| PUB-NO                 | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|------------------------|-------------------|----------|-------|------------|
| <u>NZ 524962 A</u>     | December 24, 2004 |          | 000   | A61K038/16 |
| <u>WO 200222159 A1</u> | March 21, 2002    | E        | 052   | A61K038/16 |
| <u>AU 200075771 A</u>  | March 26, 2002    |          | 000   | A61K038/16 |
| <u>EP 1322323 A1</u>   | July 2, 2003      | E        | 000   | A61K038/16 |
| <u>JP 2004508412 W</u> | March 18, 2004    |          | 095   | A61K038/00 |

INT-CL (IPC): A61 K 38/00; A61 K 38/16; A61 K 38/17; A61 K 38/19; A61 K 38/20; A61

K 39/00; A61 K 39/39; A61 K 45/00; A61 P 35/00; A61 P 35/04; A61 P 37/04; C07 K 14/415

|      |       |          |       |        |                |      |           |  |  |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KMIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|--------|

☐ 28. Document ID: CZ 200002680 A3

L6: Entry 28 of 45

File: DWPI

Mar 13, 2002

DERWENT-ACC-NO: 2002-341029

DERWENT-WEEK: 200238

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: Cyclic alkythiopeptides represents anti-oxidant compound which is protected from oxydisation by amino acid methionin - NoAbstract

INVENTOR: FRIC, P; KASAFIREK, E ; TOMASOVA, H

PRIORITY-DATA: 2000CZ-0002680 (July 21, 2000)

PATENT-FAMILY:

| PUB-NO          | PUB-DATE       | LANGUAGE | PAGES | MAIN-IPC   |
|-----------------|----------------|----------|-------|------------|
| CZ 200002680 A3 | March 13, 2002 |          | 000   | C07K005/12 |

INT-CL (IPC): A61 K 38/12; A61 P 9/10; A61 P 11/00; A61 P 25/16; A61 P 25/28; A61 P 27/12; A61 P 29/00; C07 K 5/12

|      |       |          |       |        |                |      |           |  |  |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KMIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|--------|

☐ 29. Document ID: US 6770750 B2, EP 1101771 A1, US 6300489 B1, US 20030096985 A1

L6: Entry 29 of 45

File: DWPI

Aug 3, 2004

DERWENT-ACC-NO: 2001-357927

DERWENT-WEEK: 200451

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TITLE: New pathogen induced genes (pepper defensin protein gene and pepper thionin-like protein gene) from Capsicum annuum, useful for producing transgenic plants with enhanced resistance against phytopathogens, e.g. fungi or nematode

INVENTOR: CHUNG, C H; KO, M K ; OH, B ; SHIN, B ; KYUNG KO, M

PRIORITY-DATA: 1999EP-0309059 (November 15, 1999), 1999US-0442631 (November 18, 1999), 2001US-0854562 (May 15, 2001)

PATENT-FAMILY:

| PUB-NO            | PUB-DATE        | LANGUAGE | PAGES | MAIN-IPC    |
|-------------------|-----------------|----------|-------|-------------|
| US 6770750 B2     | August 3, 2004  |          | 000   | C07H019/04  |
| EP 1101771 A1     | May 23, 2001    | E        | 022   | C07K014/415 |
| US 6300489 B1     | October 9, 2001 |          | 000   | C07H021/04  |
| US 20030096985 A1 | May 22, 2003    |          | 000   | C07H021/02  |

INT-CL (IPC): A01 N 43/04; A61 K 31/70; C07 H 19/04; C07 H 21/02; C07 H 21/04; C07 K 14/415; C12 N 15/00; C12 N 15/09; C12 N 15/63; C12 N 15/70; C12 N 15/74; C12 N 15/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | RWC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|-----|----------|

☐ 30. Document ID: WO 200133192 A1, AU 200112421 A

L6: Entry 30 of 45

File: DWPI

May 10, 2001

DERWENT-ACC-NO: 2001-355359

DERWENT-WEEK: 200364

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TITLE: Cytological staining solution for staining cells useful for cytological or histological analysis, comprises methanol, phenol and thionin

INVENTOR: ISENSTEIN, L M; LAPEN, D C ; MUI, K K ; SOULE, N W ; ZAHNISER, D J

PRIORITY-DATA: 1999US-0430198 (October 29, 1999), 1999US-0430116 (October 29, 1999), 1999US-0430117 (October 29, 1999), 1999US-0430196 (October 29, 1999)

PATENT-FAMILY:

| PUB-NO                 | PUB-DATE     | LANGUAGE | PAGES | MAIN-IPC   |
|------------------------|--------------|----------|-------|------------|
| <u>WO 200133192 A1</u> | May 10, 2001 | E        | 044   | G01N001/30 |
| <u>AU 200112421 A</u>  | May 14, 2001 |          | 000   | G01N001/30 |

INT-CL (IPC): G01 N 1/30

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | RWC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|-----|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|-----|----------|

☐ 31. Document ID: JP 2004041209 A, EP 902089 A2, JP 11075594 A, AU 9883160 A, CN 1214195 A, AU 718210 B, US 6187995 B1, EP 902089 B1, DE 69820180 E

L6: Entry 31 of 45

File: DWPI

Feb 12, 2004

DERWENT-ACC-NO: 1999-169239

DERWENT-WEEK: 200413

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TITLE: Disease-resistant transgenic plants - expressing a thionin gene from Avena sativa

INVENTOR: HIROCHIKA, H; HONKURA, R ; IWAI, T ; MITSUHARA, I ; NAKAMURA, S ; OHASHI, Y ; OHSHIMA, M ; UGAKI, M

PRIORITY-DATA: 1997JP-0243229 (September 8, 1997), 2003JP-0273117 (July 10, 2003)

PATENT-FAMILY:

| PUB-NO                 | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|------------------------|-------------------|----------|-------|------------|
| <u>JP 2004041209 A</u> | February 12, 2004 |          | 017   | A01H005/00 |
| <u>EP 902089 A2</u>    | March 17, 1999    | E        | 018   | C12N015/82 |
| <u>JP 11075594 A</u>   | March 23, 1999    |          | 012   | A01H005/00 |

|               |                   |       |            |
|---------------|-------------------|-------|------------|
| AU 9883160 A  | March 18, 1999    | 000   | A01H005/00 |
| CN 1214195 A  | April 21, 1999    | 000   | A01H001/00 |
| AU 718210 B   | April 13, 2000    | 000   | A01H005/00 |
| US 6187995 B1 | February 13, 2001 | 000   | C12N015/82 |
| EP 902089 B1  | December 3, 2003  | E 000 | C12N015/82 |
| DE 69820180 E | January 15, 2004  | 000   | C12N015/82 |

INT-CL (IPC): A01 H 1/00; A01 H 5/00; A01 N 65/00; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/63; C12 N 15/82

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | FIGS | Draw. Ds |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|

☐ 32. Document ID: WO 9902960 A1, JP 2001509591 W, AU 9882029 A, US 5942410 A, EP 996852 A1

L6: Entry 32 of 45

File: DWPI

Jan 21, 1999

DERWENT-ACC-NO: 1999-121092

DERWENT-WEEK: 200147

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TITLE: DNA staining composition with improved stability - comprises a cationic stain, especially thionin, ametabisulfite and an aqueous alcoholic solution comprising methanol or ethanol

INVENTOR: GARNER, D M; LAM, P P ; PALCIC, B ; PAYNE, P W ; PAYNE, P

PRIORITY-DATA: 1997US-0888434 (July 7, 1997)

PATENT-FAMILY:

| PUB-NO          | PUB-DATE         | LANGUAGE | PAGES | MAIN-IPC   |
|-----------------|------------------|----------|-------|------------|
| WO 9902960 A1   | January 21, 1999 | E        | 023   | G01N001/30 |
| JP 2001509591 W | July 24, 2001    |          | 025   | G01N033/48 |
| AU 9882029 A    | February 8, 1999 |          | 000   |            |
| US 5942410 A    | August 24, 1999  |          | 000   | A61K009/44 |
| EP 996852 A1    | May 3, 2000      | E        | 000   | G01N001/30 |

INT-CL (IPC): A61 K 9/44; A61 K 31/54; C09 B 55/00; C12 Q 1/68; G01 N 1/30; G01 N 33/48; G01 N 33/50; G01 N 33/53

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | FIGS | Draw. Ds |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|

☐ 33. Document ID: US 5703049 A

L6: Entry 33 of 45

File: DWPI

Dec 30, 1997

DERWENT-ACC-NO: 1998-076460

DERWENT-WEEK: 199807

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: Killing and inhibiting phytopathogenic microorganisms - by expressing methionine rich alpha-hordothionin, useful in, e.g. improving plant feed formulations

INVENTOR: RAO, A G

PRIORITY-DATA: 1996US-0608786 (February 29, 1996)

## PATENT-FAMILY:

| PUB-NO       | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|--------------|-------------------|----------|-------|------------|
| US 5703049 A | December 30, 1997 |          | 006   | A61K038/00 |

INT-CL (IPC): A61 K 38/00

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|--------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|--------|

☐ 34. Document ID: JP 09241252 A

L6: Entry 34 of 45

File: DWPI

Sep 16, 1997

DERWENT-ACC-NO: 1997-539743

DERWENT-WEEK: 199750

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TITLE: New 3,4,6,7-tetra:hydro-1H-5,2-benzoxathionin derivatives - used as angiotensin converting enzyme and acid secretion inhibitors

PRIORITY-DATA: 1996JP-0070887 (March 4, 1996)

## PATENT-FAMILY:

| PUB-NO        | PUB-DATE           | LANGUAGE | PAGES | MAIN-IPC   |
|---------------|--------------------|----------|-------|------------|
| JP 09241252 A | September 16, 1997 |          | 004   | C07D327/02 |

INT-CL (IPC): A61 K 31/38; C07 D 327/02

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|--------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|--------|------|--------|

☐ 35. Document ID: WO 9641608 A2, WO 9641608 A3, AU 9659817 A

L6: Entry 35 of 45

File: DWPI

Dec 27, 1996

DERWENT-ACC-NO: 1997-065280

DERWENT-WEEK: 199722

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TITLE: New target specific toxins, partic for cancer cells - comprising a molecule capable of specific binding to the surface of a cell linked to Pyrularia thionin peptide.

INVENTOR: GASANOV, S E; RAEL, E D ; VERNON, L P ,

PRIORITY-DATA: 1995US-0479799 (June 7, 1995)

## PATENT-FAMILY:

| PUB-NO        | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|---------------|-------------------|----------|-------|------------|
| WO 9641608 A2 | December 27, 1996 | E        | 052   | A61K000/00 |
| WO 9641608 A3 | February 6, 1997  |          | 000   | A61K000/00 |

AU 9659817 A

January 9, 1997

000

A61K039/00

INT-CL (IPC): A61 K 0/00; A61 K 39/00

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|------|----------|

☐ 36. Document ID: CN 1192239 A, WO 9638563 A1, AU 9659611 A, EP 832235 A1, US 5885802 A, BR 9609299 A, JP 11506329 W, HU 9900878 A2, AU 707354 B, MX 9709352 A1

L6: Entry 36 of 45

File: DWPI

Sep 2, 1998

DERWENT-ACC-NO: 1997-034376

DERWENT-WEEK: 200276

COPYRIGHT 2005 DERWENT INFORMATION LTD

TITLE: New modified alpha-hordothionin having methionine amino acid substns. - to increase the methionine content of e.g. animal feed

INVENTOR: RAO, G A; RAO, A G

PRIORITY-DATA: 1995US-0460440 (June 2, 1995), 1997US-0824382 (March 26, 1997)

## PATENT-FAMILY:

| PUB-NO               | PUB-DATE          | LANGUAGE | PAGES | MAIN-IPC   |
|----------------------|-------------------|----------|-------|------------|
| <u>CN 1192239 A</u>  | September 2, 1998 |          | 000   | C12N015/29 |
| <u>WO 9638563 A1</u> | December 5, 1996  | E        | 021   | C12N015/29 |
| <u>AU 9659611 A</u>  | December 18, 1996 |          | 000   | C12N015/29 |
| <u>EP 832235 A1</u>  | April 1, 1998     | E        | 000   | C12N015/29 |
| <u>US 5885802 A</u>  | March 23, 1999    |          | 000   | C12N015/09 |
| <u>BR 9609299 A</u>  | May 11, 1999      |          | 000   | C12N015/29 |
| <u>JP 11506329 W</u> | June 8, 1999      |          | 020   | C12N015/09 |
| <u>HU 9900878 A2</u> | July 28, 1999     |          | 000   | C12N015/29 |
| <u>AU 707354 B</u>   | July 8, 1999      |          | 000   | C12N015/29 |
| <u>MX 9709352 A1</u> | February 1, 1998  |          | 000   | C12N015/09 |

INT-CL (IPC): A01 H 1/00; A01 H 5/00; C07 K 14/00; C07 K 14/415; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/82; C12 P 21/02; C12 P 21/02; C12 R 1:91

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Abstract | Abstract | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|----------|----------|--------|------|----------|

☐ 37. Document ID: CN 1192238 A, WO 9638562 A1, AU 9659610 A, EP 828835 A1, US 5885801 A, BR 9609200 A, AU 705933 B, HU 9900876 A2, JP 11511007 W, MX 9709351 A1

L6: Entry 37 of 45

File: DWPI

Sep 2, 1998

DERWENT-ACC-NO: 1997-034375

DERWENT-WEEK: 200276

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TITLE: New modified alpha-hordothionin having threonine amino acid substns. - to increase the threonine content of e.g. animal feed

INVENTOR: RAO, G A; RAO, A G

PRIORITY-DATA: 1995US-0459180 (June 2, 1995), 1997US-0824379 (March 26, 1997)

## PATENT-FAMILY:

| PUB-NO        | PUB-DATE           | LANGUAGE | PAGES | MAIN-IPC   |
|---------------|--------------------|----------|-------|------------|
| CN 1192238 A  | September 2, 1998  |          | 000   | C12N015/29 |
| WO 9638562 A1 | December 5, 1996   | E        | 019   | C12N015/29 |
| AU 9659610 A  | December 18, 1996  |          | 000   | C12N015/29 |
| EP 828835 A1  | March 18, 1998     | E        | 000   | C12N015/29 |
| US 5885801 A  | March 23, 1999     |          | 000   | C12N015/09 |
| BR 9609200 A  | May 11, 1999       |          | 000   | C12N015/29 |
| AU 705933 B   | June 3, 1999       |          | 000   | C12N015/29 |
| HU 9900876 A2 | July 28, 1999      |          | 000   | C12N015/29 |
| JP 11511007 W | September 28, 1999 |          | 018   | C12N015/09 |
| MX 9709351 A1 | February 1, 1998   |          | 000   | C12N015/29 |

INT-CL (IPC): A01 H 1/00; A01 H 5/00; C07 K 14/00; C07 K 14/415; C12 N 1/21; C12 N 5/10; C12 N 15/09; C12 N 15/29; C12 N 15/82; C12 P 13/08; C12 P 13/08; C12 R 1:91

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|

☐ 38. Document ID: JP 08266279 A, JP 2775405 B2

L6: Entry 38 of 45

File: DWPI

Oct 15, 1996

DERWENT-ACC-NO: 1996-512666

DERWENT-WEEK: 199833

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TITLE: DNA encoding novel Avena sativa L derived thionin - which can be introduced into plant to confer high antimicrobial activity

PRIORITY-DATA: 1995JP-0074158 (March 30, 1995)

## PATENT-FAMILY:

| PUB-NO        | PUB-DATE         | LANGUAGE | PAGES | MAIN-IPC   |
|---------------|------------------|----------|-------|------------|
| JP 08266279 A | October 15, 1996 |          | 011   | C12N015/09 |
| JP 2775405 B2 | July 16, 1998    |          | 011   | C12N015/09 |

INT-CL (IPC): A01 H 5/00; A01 N 63/00; A61 K 38/00; C07 H 21/04; C07 K 14/415; C12 N 5/10; C12 N 15/09; C12 P 21/02; C12 P 21/02; C12 R 1:91; C12 N 5/10; C12 R 1:91; C12 P 21/02; C12 R 1:91

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|----------|

☐ 39. Document ID: WO 9416078 A1, DE 69428290 E, AU 9461622 A, WO 9416078 A3, EP 745126 A1, US 5990389 A, CA 2161881 C, EP 745126 B1

L6: Entry 39 of 45

File: DWPI

Jul 21, 1994

DERWENT-ACC-NO: 1994-249225

DERWENT-WEEK: 200169

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TITLE: Derivatives of alpha hordothionin - have high lysine content, and retain antifungal activity of parent compound

INVENTOR: BEACH, L R; RAO, A G ; BEACH, L ; RAO, A

PRIORITY-DATA: 1993US-0003885 (January 13, 1993), 1995US-0369975 (January 6, 1995), 1995US-0575654 (December 20, 1995), 1997US-0838763 (April 10, 1997)

## PATENT-FAMILY:

| PUB-NO               | PUB-DATE           | LANGUAGE | PAGES | MAIN-IPC   |
|----------------------|--------------------|----------|-------|------------|
| <u>WO 9416078 A1</u> | July 21, 1994      | E        | 027   | C12N015/29 |
| <u>DE 69428290 E</u> | October 18, 2001   |          | 000   | C12N015/29 |
| <u>AU 9461622 A</u>  | August 15, 1994    |          | 000   | C12N015/29 |
| <u>WO 9416078 A3</u> | September 1, 1994  |          | 000   | C12N015/29 |
| <u>EP 745126 A1</u>  | December 4, 1996   | E        | 000   | C12N015/29 |
| <u>US 5990389 A</u>  | November 23, 1999  |          | 000   | A01H005/00 |
| <u>CA 2161881 C</u>  | March 27, 2001     | E        | 000   | C12N015/29 |
| <u>EP 745126 B1</u>  | September 12, 2001 | E        | 000   | C12N015/29 |

INT-CL (IPC): A01 G 13/00; A01 H 5/00; A01 N 37/18; A01 N 43/36; A01 N 65/00; C07 K 13/00; C07 K 14/00; C07 K 14/415; C12 N 1/21; C12 N 5/10; C12 N 15/29; C12 N 15/82

|      |       |          |       |        |                |      |           |  |  |        |      |          |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|

☐ 40. Document ID: JP 06070694 A

L6: Entry 40 of 45

File: DWPI

Mar 15, 1994

DERWENT-ACC-NO: 1994-123292

DERWENT-WEEK: 199415

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TITLE: Cpd. feed for flatfish using vegetable protein - comprises soybean protein-contg. vegetable protein, methionin and lysine

PRIORITY-DATA: 1992JP-0254129 (August 29, 1992)

## PATENT-FAMILY:

| PUB-NO               | PUB-DATE       | LANGUAGE | PAGES | MAIN-IPC   |
|----------------------|----------------|----------|-------|------------|
| <u>JP 06070694 A</u> | March 15, 1994 |          | 007   | A23K001/14 |

INT-CL (IPC): A23K 1/14; A23K 1/16; A23K 1/18

|      |       |          |       |        |                |      |           |  |  |        |      |          |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|

☐ 41. Document ID: FR 2183550 A, SU 688117 A

L6: Entry 41 of 45

File: DWPI

Jan 25, 1974



DERWENT-ACC-NO: 1974-11878V

DERWENT-WEEK: 200394

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TITLE: Deodourising residual sodium sulphate - from ethionin mfr by treatment with hydrogen peroxide stabilised with alkali phosphate

PRIORITY-DATA: 1972FR-0016637 (May 10, 1972)

## PATENT-FAMILY:

| PUB-NO              | PUB-DATE           | LANGUAGE | PAGES | MAIN-IPC |
|---------------------|--------------------|----------|-------|----------|
| <u>FR 2183550 A</u> | January 25, 1974   |          | 000   |          |
| <u>SU 688117 A</u>  | September 25, 1979 |          | 000   |          |

INT-CL (IPC): C01D 5/00

|      |       |          |       |        |                |      |           |  |  |        |      |          |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|

☐ 42. Document ID: JP 48044491 A, JP 81019998 B

L6: Entry 42 of 45

File: DWPI

DERWENT-ACC-NO: 1973-57061U

DERWENT-WEEK: 200402

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TITLE: Simultaneous s-adenosylmethionine and glutathione - fermentative prodn - by culture of sacchoromyces yeasts on melthionin

PRIORITY-DATA: 1971JP-0079411 (October 11, 1971)

## PATENT-FAMILY:

| PUB-NO               | PUB-DATE     | LANGUAGE | PAGES | MAIN-IPC |
|----------------------|--------------|----------|-------|----------|
| <u>JP 48044491 A</u> |              |          | 000   |          |
| <u>JP 81019998 B</u> | May 11, 1981 |          | 000   |          |

INT-CL (IPC): C12P 19/18; C12P 21/02; C12R 1/85

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|------|-------|----------|-------|--------|----------------|------|-----------|--|--|--------|------|----------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference |  |  | Claims | KWIC | Draw. De |
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☐ 43. Document ID: US 3299035 A

L6: Entry 43 of 45

File: USOC

Jan 17, 1967

US-PAT-NO: 3299035

DOCUMENT-IDENTIFIER: US 3299035 A

TITLE: L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucyl-glycyl-L-leucyl-L-methionin-amide and its acid addition salts

DATE-ISSUED: January 17, 1967

INVENTOR-NAME: EDMOND SANDRIN; ROGER BOISSONNAS

US-CL-CURRENT: 530/329, 930/10, 930/20

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|
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☐ 44. Document ID: US 2794786 A

L6: Entry 44 of 45

File: USOC

Jun 4, 1957

US-PAT-NO: 2794786

DOCUMENT-IDENTIFIER: US 2794786 A

TITLE: Thionin dye-ion exchange resin indicator compounds

DATE-ISSUED: June 4, 1957

INVENTOR-NAME: SEGAL HARRY L; MILLER LEON L

US-CL-CURRENT: 521/29, 436/101, 521/32, 521/33

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|--------|------|--------|
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☐ 45. Document ID: US 2014519 A

L6: Entry 45 of 45

File: USOC

Sep 17, 1935

US-PAT-NO: 2014519

DOCUMENT-IDENTIFIER: US 2014519 A

TITLE: Argento-chrome tetramethylthionin and process of manufacture

DATE-ISSUED: September 17, 1935

INVENTOR-NAME: JULIAN BLOCK DAVID

US-CL-CURRENT: 544/4; 987/22

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D |
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L5: Entry 1 of 28

File: PGPB

Jan 13, 2005

DOCUMENT-IDENTIFIER: US 20050009781 A1

TITLE: Methods of skin treatment and use of water-soluble beta-(1,3) glucans as active agents for producing therapeutic skin treatment agents

Detail Description Paragraph:

[0073] In addition to the primary light protection substances also secondary light protection substances of the antioxidant type find use, which interrupt the photochemical reaction chain, which is initiated when UV radiation penetrates the skin. Typical examples of such are amino acids (e.g. glycine, histidine, tyrosine, tryptophan) and their derivatives, imidazoles (e.g. urocanic acid) and their derivatives, peptides such as D,L-carnosine, D-carnosine, L-carnosine and their derivatives (e.g. anserine), carotinoids, carotene (e.g. .alpha.-carotin, .beta.-carotin, lycopene) and their derivatives, chlorogenic acid and its derivatives, liponic acid and its derivatives (e.g. dihydroliponic acid), aurothioglucose, propylthiouracil and other thiols (e.g. thioredoxin, glutathione, cysteine, cystine, cystamine and their glycosyl, n-acetyl, methyl, ethyl, propyl, amyl, butyl and lauryl, palmitoyl, oleyl, .gamma.-linoleyl, cholesteryl and glyceryl esters) as well as their salts, dilauryl thiodipropionate, distearyl thiodipropionate, thiodipropionic acid and their derivatives (esters, ethers, peptides, lipides, nucleotides, nucleosides and salts) as well as sulfoximine compounds (e.g. buthionin sulfoximines, homocysteine sulfoximines, butionin sulfones, penta-, hexa-, hepta-thionin sufoximine) in very small compatible doses (e.g. pmol to .mu.mol/kg), further (metal) chelating agents (e.g. .alpha.-hydroxy fatty acids, palmitic acid, phytic acid, lactoferrin), .alpha.-hydroxy acids (e.g. citric acid, lactic acid, malic acid), humic acid, gallic acid, gallic extracts, bilirubin, bifiverdin, EDTA, EGTA and their derivatives, unsaturated fatty acids and their derivatives (e.g. .gamma.-linolenic acid, linolic acid, oleic acid), folic acid and their derivatives, ubiquinol and ubiquinol and their derivatives, vitamin C and derivatives (e.g. ascorbyl palmitate, Mg-ascorbyl phosphate, ascorbyl acetate), tocopherols and derivatives (e.g. vitamin E acetate), vitamin A and derivatives (vitamin A palmitate) as well as koniferyl benzoate of benzoin resin, rutinic acid and their derivatives, .alpha.-glycosylrutin, ferulic acid, furfurylidene glucitol, carnosine, butylhydroxy toluene, butylhydroxy anisole, nordihydro guaiac resin acid, nordihydro guaiacetic acid, trihydroxy butyrophenone, uric acid and their derivatives, mannose and its derivatives, super oxide dismutase, zinc and its derivatives (e.g. ZnO, ZnSO<sub>4</sub>), selenium and its derivatives (e.g. selen-methionine), stilbenes and their derivatives (e.g. stilbene oxide, trans-stilbene oxide) and the derivatives suitable according to the invention (salts, esters, ethers, sugars, nucleotides, nucleosides, peptides and lipids) of these mentioned active substances.

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L5: Entry 2 of 28

File: PGPB

May 6, 2004

DOCUMENT-IDENTIFIER: US 20040087530 A1

TITLE: Methods and compositions for modulating leptin activity

Detail Description Paragraph:

[0094] In order to localize the specific anatomic regions of the hypothalamus and other parts of the brain in which leptin affects SOCS-3 mRNA levels, .sup.35S-labeled RNA antisense probe was generated. The SOCS-3A and SOCS-3B primers from above were used amplify a 450 base pair fragment of the mouse SOCS-3 CDNA. The PCR products were cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) according to the manufactures recommendations. The orientation of the cloned CDNA was verified by sequencing using standard double-stranded plasmid techniques. For generation of sense .sup.35S-labeled RNA, the plasmid was linearized by digestion with BamHI, and subjected to in vitro transcription with T7 polymerase according to the manufactures protocols (Promega). In situ hybridization histochemistry was conducted according to methods well known in the art (Simmons). Tissue sections of mouse and rat brain were mounted onto slides, air dried, and stored in desiccated boxes at -20.degree. C. Prior to hybridization, the slides were immersed in 10% neutral buffered formalin, incubated in 0.001% proteinase K (Boehringer Mannheim) for 30 min., then in 0.025% acetic anhydride for 10 min., and dehydrated in ascending concentrations ethanol. The RNA probes were then diluted to 10.sup.6 cpm/ml in hybridization solution of 50% formamide, 10 mM Tris-HCl, pH 8.0, 5 mg tRNA, 10 mM dithiothreitol, 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA, pH 8, and 1.times. Denhardt's solution (Sigma). Hybridization solution and a glass coverslip was applied to each slide and sections were then incubated for 12-16 hours at 56.degree. C. The coverslips were removed and the slides washed 4 times with 4.times.SSC. Sections were then incubated in 0.002% RNAase A (Boehringer Mannheim) with 0.5 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA, for 30 min. at 37.degree. C. Sections were rinsed in decreasing concentrations of SSC containing 0.25% DTT: 2.times. at 50.degree. C. for 1 hour, 0.2.times. at 55.degree. C. for 1 hour, and 0.2.times. for 1 hour at 60.degree. C. Sections were next dehydrated in graded ethanol (50, 70, 80, and 90%) containing 0.3 M NH.sub.4OAc followed by 100% ethanol. Slides were air dried and placed in X-ray film cassettes with BMR-2 film (Kodak) for 3-5 days. Slides were then dipped in NTB2 photographic emulsion (Kodak), dried and stored with desiccant in foil-wrapped slide boxes at 4.degree. C. for 2-3 weeks. Slides were developed with D-19 developer (Kodak); counterstained with thionin, dehydrated in graded ethanol, cleared in xylene, and coverslipped with with Permaslip. Sections were analyzed with a Zeiss Axioplan light microscope using brightfield and darkfield optics. Photomicrographs were produced by capturing images with a digital camera (Kodak, DCS) mounted directly on the microscope and an Apple Macintosh Power PC computer. Image editing software (Adobe Photoshop) was used to combine photomicrographs into plates and figures were printed on a dye sublimation printer (Kodak 8600). Only the sharpness, contrast, and brightness were adjusted. The results are shown in FIG. 2. In brain sections from normal rats fed ad libitum and given a single intravenous injection of recombinant leptin (1 .mu.g/g body weight), strong specific hybridization was detected in the arcuate nucleus (Arc) and the dorsomedial hypothalamic nucleus (DMH), as compared to saline injected rat brain sections (FIGS. 2B and 2A, respectively). In other regions of the brain, including the cerebellum, no specific hybridization signals were detected.

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May 15, 2003

TITLE: Bactericidal composition containing peptide and chelating agent

A bactericidal composition is provided, which comprises as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin. The bactericidal composition, for example, has the effect of sterilizing food poisoning bacteria at a low concentration and is highly safe.

[0011] The first aspect of the present invention relates to a bactericidal composition comprising as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid (sometimes referred to as EDTA hereinafter) and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

[0013] The third aspect of the present invention relates to a bactericidal composition according to the second aspect of the present invention, in which the content of the above (a) at least one substance selected from the group consisting of EDTA and metal salts thereof is 0.05 mM or more and less than 1.5 mM and the content of the above (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin is 1 .mu.g/mL or more and 150 .mu.g/mL or less.

[0014] FIG. 1 is a graph illustrating minimum bactericidal concentration (MBC) of alpha-type thionin of wheat when food poisoning bacteria are cultivated at 37.degree. C. for 1 day in an LB medium containing EDTA-2Na in a varied concentration.

[0015] The bactericidal composition of the present invention contains at least one substance selected from the group consisting of EDTA and metal salts thereof and at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin as effective ingredients.

[0027] The bactericidal composition of the present invention may be formulated into various forms. For example, EDTA or its metal salt and thionin may be simultaneously simultaneously added to various foods. EDTA and its metal salts as well as thionins have heat resistance, so that they may be added to food prior to pasteurize the food.

[0028] To sterilize food poisoning bacteria contained in food, it may be advantageous to simultaneously add 0.1 to 0.5 mM EDTA or its metal salt and 2 to

100 .mu.g/mL thionin for *V. parahaemolyticus*, 0.1 to 1.49 mM EDTA or its metal salt and 2 to 100 .mu.g/mL thionin for *Salmonella* sp., or 0.5 to 1.49 mM EDTA or its metal salt and 10 to 100 .mu.g/mL thionin for *E. coli*. However, the lower the concentration of EDTA or its metal salt is, the higher the concentration of thionin to be added must be.

Detail Description Paragraph:

[0030] According to the present invention, there is provided a bactericidal composition comprising as active ingredients (a) at least one substance selected from the group consisting of EDTA and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

Detail Description Paragraph:

[0032] Aqueous solutions of EDTA or its metal salts and of thionin are each colorless, transparent and odorless, so that they give no influence on the taste of food and so forth.

Detail Description Paragraph:

[0040] Then, after adding predetermined amounts of thionin and EDTA, the bacteria were inoculated so that the concentration of bacteria was about 1.times.10.sup.6 cells/mL. After cultivating the bacteria for 1 day for other bacteria, the viable count was measured by a method of diluted plate culture. The concentration in which the viable count was decreased to {fraction (1/100)} or less was defined as minimum bactericidal concentration (MBC) and the concentration in which the viable count was not increased was defined as minimum inhibitory concentration (MIC). The results obtained are shown in Tables 1, 2 and 3, as well as in FIG. 1.

Detail Description Paragraph:

[0041] As will be clear from Table 1, when alpha-type thionin of wheat was added alone, the test bacteria except for *Salmonella typhimurium* JCM 6977 and *V. parahaemolyticus* IFO 12711 showed an MIC of 50 .mu.g/mL or more. On the other hand, when EDTA-2Na was added alone, *Salmonella* sp. and *E. coli* had an MBC of 10 mM or more whereas other bacteria had an MBC of 1 mM or less.

Detail Description Paragraph:

[0042] Next, 10 .mu.g/mL of alpha-type thionin of wheat and 0.02 to 10 mM of EDTA-2Na were added simultaneously. In the case of food poisoning bacteria, the MBC of EDTA-2Na was decreased as compared with the addition of EDTA-2Na alone.

Detail Description Paragraph:

[0044] Furthermore, the MBC of alpha-type thionin of wheat on *S. typhimurium* JCM 6977, *E. coli* JCM 5491 and *V. parahaemolyticus* IFO 12711 in the presence of EDTA-2Na in different concentrations was examined, and the results as shown in FIG. 1 were obtained. The tests were carried out by cultivation in an LB medium at 37.degree. C. for 1 day. In FIG. 1, the symbol .circle-solid. indicates *S. typhimurium* JCM 6977, .largecircle. indicates *E. coli* JCM 5491 and .quadrature. indicates *V. parahaemolyticus* IFO 12711. Concerning the antibacterial activity of thionin in the coexistence of EDTA-2Na, substantially no difference was observed between the thionin derived from wheat and the thionin derived from barley, and also also between the alpha-type and the beta-type (Table 3).

Detail Description Table CWU:

1 TABLE 1 MIC MBC Thionin\*.sup.1 EDTA-2Na Thionin + Strain (.mu.g/mL) (mM) EDTA-2Na\*.sup.2 *Salmonella typhimurium* 30 20 1 JCM 6977 *Salmonella enteritidis* 100< 50< 1 IFO 3313 *Escherichia coli* 01:K1:H7 100< 50 10 JCM 1649 *Escherichia coli* 06 100 10 1 JCM 5491 *Vibrio parahaemolyticus* 20 1 0.1 IFO 12711 *Bacillus cereus* 100 0.5 0.02 JCM 2152 *Bacillus cereus* 100< 0.2 0.05 IFO 15305 \*.sup.1 Alpha-type thionin of wheat \*.sup.2 MBC (mM) of EDTA-2Na in the coexistence of 10 .mu.g/mL of wheat alpha-type thionin

2TABLE 2 Thionin\* MBC (nM) Strain (10 .mu.g/mL) EDTA EDTA-2Na EDTA-4Na S.  
typhimurium - 5 20 50< JCM 6977 + 0.1 1 1 E. coli - 5 10 10 JCM 5491 + 1 1 1  
\*Alpha-type thionin of wheat

1. A bactericidal composition comprising as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin.

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File: PGPB

Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142466 A1

TITLE: Methods and compositions for modulating ciliary neurotrophic factor activity

Detail Description Paragraph:

[0116] In order to localize the specific anatomic regions of the hypothalamus and other parts of the brain in which leptin affects SOCS-3 mRNA levels, .sup.35S-labeled RNA antisense probe was generated. The SOCS-3 A and SOCS-3B primers from above were used amplify a 450 base pair fragment of the mouse SOCS-3 cDNA. The PCR products were cloned into pCR2.1 (Invitrogen, Carlsbad, Calif.) according to the manufactures recommendations. The orientation of the cloned cDNA was verified by sequencing using standard double-stranded plasmid techniques. For generation of sense .sup.35S-labeled RNA, the plasmid was linearized by digestion with BamHI, and subjected to in vitro transcription with T7 polymerase according to the manufactures protocols (Promega). In situ hybridization histochemistry was conducted according to methods well known in the art (Simmons). Tissue sections of mouse and rat brain were mounted onto slides, air dried, and stored in desiccated boxes at -20.degree. C. Prior to hybridization, the slides were immersed in 10% neutral buffered formalin, incubated in 0.001% proteinase K (Boehringer Mannheim) for 30 min., then in 0.025% acetic anhydride for 10 min., and dehydrated in ascending concentrations ethanol. The RNA probes were then diluted to 10.sup.6 cpm/ml in hybridization solution of 50% formamide, 10 mM Tris-HCl, pH 8.0, 5 mg tRNA, 10 mM dithiothreitol, 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA, pH 8, and 1.times. Denhardt's solution (Sigma). Hybridization solution and a glass coverslip was applied to each slide and sections were then incubated for 12-16 hours at 56.degree. C. The coverslips were removed and the slides washed 4 times with 4.times. SSC. Sections were then incubated in 0.002% RNAase A (Boehringer Mannheim) with 0.5 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA, for 30 min. at 37.degree. C. Sections were rinsed in decreasing concentrations of SSC containing 0.25% DTT: 2.times. at 50.degree. C. for 1 hour, 0.2.times. at 55.degree. C. for 1 hour, and 0.2.times. for 1 hour at 60.degree. C. Sections were next dehydrated in graded ethanol (50, 70, 80, and 90%) containing 0.3 M NH.sub.4OAc followed by 100% ethanol. Slides were air dried and placed in X-ray film cassettes with BMR-2 film (Kodak) for 3-5 days. Slides were then dipped in NTB2 photographic emulsion (Kodak), dried and stored with desiccant in foil-wrapped slide boxes at 4.degree. C. for 2-3 weeks. Slides were developed with D-19 developer (Kodak), counterstained with thionin, dehydrated in graded ethanol, cleared in xylene, and coverslipped with Permaslip. Sections were analyzed with a Zeiss Axioplan light microscope using brightfield and darkfield optics. Photomicrographs were produced by capturing images with a digital camera (Kodak, DCS) mounted directly on the microscope and an Apple Macintosh Power PC computer. Image editing software (Adobe Photoshop) was used to combine photomicrographs into plates and figures were printed on a dye sublimation printer (Kodak 8600). Only the sharpness, contrast, and brightness were adjusted.

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L5: Entry 13 of 28

File: USPT

Dec 31, 2002

DOCUMENT-IDENTIFIER: US 6500410 B1

TITLE: Sun protection agents

Brief Summary Text (41):

In addition to the primary light protection substances also secondary light protection substances of the antioxidant type find use, which interrupt the photochemical reaction chain, which is initiated when UV radiation penetrates the skin. Typical examples of such are amino acids (e.g. glycine, histidine, tyrosine, tryptophan) and their derivatives, imidazoles (e.g. urocanic acid) and their derivatives, peptides such as D,L-carnosine, D-carnosine, L-carnosine and their derivatives (e.g. anserine), carotinoids, carotene (e.g. .alpha.-carotin, .beta.-carotin, lycopene) and their derivatives, chlorogenic acid and its derivatives, liponic acid and its derivatives (e.g. dihydroliponic acid), aurothioglucose, propylthiouracil and other thiols (e.g. thioredoxin, glutathione, cysteine, cystine, cystamine and their glycosyl, n-acetyl, methyl, ethyl, propyl, amyl, butyl and lauryl, palmitoyl, oleyl, .gamma.-linoleyl, cholesteryl and glyceryl esters) as well as their salts, dilauryl thiodipropionate, distearyl thiodipropionate, thiodipropionic acid and their derivatives (esters, ethers, peptides, lipides, nucleotides, nucleosides and salts) as well as sulfoximine compounds (e.g. buthionin sulfoximines, homocysteine sulfoximines, butionin sulfones, penta-, hexa-, hepta-thionin sufoximine) in very small compatible doses (e.g. pmol to .mu.mol/kg), further (metal) chelating agents (e.g. .alpha.-hydroxy fatty acids, palmitic acid, phytic acid, lactoferrin), .alpha.-hydroxy acids (e.g. citric acid, lactic acid, malic acid), humic acid, gallic acid, gallic extracts, bilirubin, bifiverdin, EDTA, EGTA and their derivatives, unsaturated fatty acids and their derivatives (e.g. .gamma.-linolenic acid, linolic acid, oleic acid), folic acid and their derivatives, ubiquinol and ubiquinol and their derivatives, vitamin C and derivatives (e.g. ascorbyl palmitate, Mg-ascorbyl phosphate, ascorbyl acetate), tocopherols and derivatives (e.g. vitamin E acetate), vitamin A and derivatives (vitamin A palmitate) as well as koniferyl benzoate of benzoin resin, rutinic acid and their derivatives, .alpha.-glycosylrutin, ferulic acid, furfurylidene glucitol, carnosine, butylhydroxy toluene, butylhydroxy anisole, nordihydro guaiac resin acid, nordihydro guaiacetic acid, trihydroxy butyrophenone, uric acid and their derivatives, mannose and its derivatives, superoxide dismutase, zinc and its derivatives (e.g. ZnO, ZnSO<sub>4</sub>), selenium and its derivatives (e.g. selen-methionine), stilbenes and their derivatives (e.g. stilbene oxide, trans-stilbene oxide) and the derivatives suitable according to the invention (salts, esters, ethers, sugars, nucleotides, nucleosides, peptides and lipids) of these mentioned active substances.

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Aug 14, 2001

TITLE: L-AP4 sensitive glutamate receptors

For in situ hybridization, adult Sprague-Dawley rats (200-250 g) were anesthetized with pentobarbital and perfused transcardially with ice-cold saline, followed by ice-cold fixative (4% paraformaldehyde/0.1 M sodium borate, pH 9.5). The brains were post-fixed overnight in fixative containing 10% sucrose. Sections (25  $\mu$ m) were mounted on gelatin- and poly-L-lysine-coated glass, fixed for 15 minutes in 4% paraformaldehyde/0.1 M phosphate buffered saline (PBS), washed twice in 0.1 M PBS, and treated for 30 min at 37.degree. C. in proteinase K (0.001% in 0.1 M Tris/0.05 M EDTA, pH 8) followed by 0.0025% acetic anhydride in 0.1 M triethanolamine at room temperature followed by dehydration. A 719 bp XhoI fragment containing nucleotides 1-197 of mGluR7 and a 1234 bp XhoI/PstI fragment of mGluR4 containing nucleotides 791-2025 were subcloned into pBluescript KS(+). <sup>35</sup>S-labeled antisense RNA was transcribed from each template and used for hybridization at 10<sup>7</sup> cpm/ml for 20 hours at 58.degree. C. in hybridization buffer (Mountjoy et al., Science 257: 1248-1251 [1992]). Slides were processed as described (Simerly and Young, Mol. Endocrinol. 5: 424-432 [1991]) then dipped in NTB-2 liquid photographic emulsion (Kodak), exposed for 13 days, developed with D-19 developer and counterstained with thionin.

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L5: Entry 19 of 28

File: USPT

Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136550 A

TITLE: Neuronal nicotinic acetylcholinergic receptor compositions

Detailed Description Text (235):

Antisense [<sup>35</sup>S]-UTP-labeled RNA probes were synthesized in vitro from pESD77 and used to map the distribution of transcripts corresponding to  $\lambda$ .ESD-7 in the rat brain. Paraformaldehyde-fixed 30  $\mu$ m thick rat brain sections were mounted on polylysine coated slides, then digested with proteinase K (10 mg/ml, 37.degree. C., 30 min), acetylated and dehydrated in graded ethanol solutions. Approximately 5.times.10<sup>5</sup> cpm/ml of the RNA probe was hybridized in situ at 55.degree. C. for 12 hrs in 50% formamide, 0.3M NaCl, 10 mM Tris (pH 8), 1 mM EDTA, 0.05% tRNA, 10% dextran sulfate, IX Denhardt's solution, and 10 mM DTT. Glass cover slips were removed from tissue sections by washing in 4x SSC for 15min at room temperature. Sections were treated with RNase A (20  $\mu$ g/ml, 37.degree. C., 30 min), washed for 30 min in 2x SSC, 1 mM DTT at room temperature and for 30 min in 0.1x SSC, 1 mM DTT at 55.degree. C. Sections were dehydrated in graded ethanol solutions containing 1 mM DTT and exposed to Kodak XAR film at room temperature for 1-2 days. For higher resolution analysis slides were dipped in Kodak NTB-2 nuclear photographic emulsion, which was diluted 1:1 with distilled water, at 40.degree. C. Seven to ten days after dipping, slides were developed and stained with thionin. The distribution of silver grains was analyzed with dark field illumination.

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Dec 11, 2003

TITLE: Method for producing antimicrobial protein and fusion protein

[0076] The insoluble fractions of the fusion proteins of the Examples 1 and 2 and the Comparative Example 2 and the insoluble fraction from the culture of *Escherichia coli* BL21 (DE3) pLyss with no inserted vector therein as the control for the assay of antimicrobial activity were rinsed twice in 0.5% Triton X-100/1 mM EDTA. Subsequently, a urea solution (8 M urea, 50 mM Tris-HCl at pH 8.0, 1 mM DTT, 1 mM EDTA) was added for solubilization. After centrifugation, the supernatant was transferred to a dialysis tube, for one-hour dialysis at 4.degree. C. against 4M urea solution. During the dialysis against the urea solution containing DTT, the disulfide bond in thionin may be cleaved.

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| <input type="checkbox"/> | L1                  | thionin                                                                                      | 662                  |
| <input type="checkbox"/> | L2                  | \$thionin                                                                                    | 1116                 |
| <input type="checkbox"/> | L3                  | L2 or l1                                                                                     | 1221                 |
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| <input type="checkbox"/> | L5                  | L3 same (edta or na-edta or sodiumedta or naedta or ethylenediamine\$ or ethylene-diamine\$) | 28                   |
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| <input type="checkbox"/> | L7                  | l6 and (edta or na-edta or sodiumedta or naedta or ethylenediamine\$ ir ethylene-diamine\$)  | 0                    |
| <input type="checkbox"/> | L8                  | l6 and (edta or na-edta or sodiumedta or naedta or ethylenediamine\$ or ethylene-diamine\$)  | 0                    |
| <input type="checkbox"/> | L9                  | l1.ti. and edta                                                                              | 2                    |

END OF SEARCH HISTORY

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### Entry information

Entry name **THN1\_WHEAT**  
Primary accession number **P01544**  
Secondary accession numbers None  
Entered in Swiss-Prot in Release 01, July 1986  
Sequence was last modified in Release 26, July 1993  
Annotations were last modified in Release 46, February 2005

### Name and origin of the protein

Protein name **Alpha-1-purothionin [Precursor] [Fragment]**  
Synonym **Purothionin A-II**  
Gene name **Name: THI1.1**  
**Synonyms: PUR-D1**  
From **Triticum aestivum (Wheat) [TaxID: 4565]**  
Taxonomy **Eukaryota; Viridiplantae; Streptophyta; Embryophyta;  
Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales;  
Poaceae; Pooideae; Triticeae; Triticum.**

### References

#### [1] NUCLEOTIDE SEQUENCE.

**TISSUE**=Endosperm;  
**DOI**=10.1104/pp.106.3.1221;**MEDLINE**=95125120;**PubMed**=7824649 [NCBI, ExPASy, EBI, Israel, Japan]  
Castagnaro A., Marana C., Carbonero P., Garcia-Olmedo F.;  
"cDNA cloning and nucleotide sequences of alpha 1 and alpha 2 thionins from hexaploid wheat endosperm.";  
**Plant Physiol.** 106:1221-1222(1994).

#### [2] PROTEIN SEQUENCE OF 17-61.

**STRAIN**=cv. Manitoba 3;  
**MEDLINE**=78026451;**PubMed**=914810 [NCBI, ExPASy, EBI, Israel, Japan]  
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;  
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast

from wheat flour.";

J. Biochem. 82:753-767(1977).

[3] PROTEIN SEQUENCE OF 17-61.

Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;

"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";  
Agric. Biol. Chem. 39:2269-2270(1975).

[4] PROTEIN SEQUENCE OF 17-61.

**STRAIN**=cv. Manitou;

Jones B.L., Mak A.S.;

"Amino acid sequences of the two alpha-purothionins of hexaploid wheat.";  
Cereal Chem. 54:511-523(1977).

[5] X-RAY CRYSTALLOGRAPHY (2.5 ANGSTROMS).

MEDLINE=91045879;PubMed=2235992 [NCBI, ExPASy, EBI, Israel, Japan]

Teeter M.M., Ma X.-Q., Rao U., Whitlow M.;

"Crystal structure of a protein-toxin alpha 1-purothionin at 2.5A and a comparison with predicted models.";  
Proteins 8:118-132(1990).

**Comments**

- **FUNCTION:** Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- **SUBCELLULAR LOCATION:** Secreted.
- **SIMILARITY:** Belongs to the plant thionin (TC 1.C.44) family [view classification].

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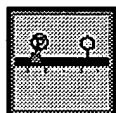
|              |                                                                  |
|--------------|------------------------------------------------------------------|
| EMBL         | X70666; CAA50004.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]  |
| PDB          | 2PLH; X-ray; @=17-60. [ExPASy / RCSB / EBI]                      |
| InterPro     | IPR001010; Thionin.<br>Graphical view of domain structure.       |
| Pfam         | PF00321; Thionin; 1.<br>Pfam graphical view of domain structure. |
| PRINTS       | PR00287; THIONIN.                                                |
| PROSITE      | PS00271; THIONIN; 1.                                             |
| BLOCKS       | P01544.                                                          |
| ProtoNet     | P01544.                                                          |
| ProtoMap     | P01544.                                                          |
| PRESAGE      | P01544.                                                          |
| DIP          | P01544.                                                          |
| ModBase      | P01544.                                                          |
| SMR          | P01544; FF7310D921C4EE30.                                        |
| SWISS-2DPAGE | Get region on 2D PAGE.                                           |
| UniRef       | View cluster of proteins with at least 50% / 90% identity.       |

**Keywords**

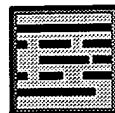
**3D-structure; Direct protein sequencing; Plant defense; Plant toxin; Signal; Thionin.**

**Features**





Feature table viewer



Feature aligner

| Key      | From | To  | Length | Description          |
|----------|------|-----|--------|----------------------|
| NON_TER  | 1    | 1   |        |                      |
| SIGNAL   | <1   | 16  | >16    |                      |
| CHAIN    | 17   | 61  | 45     | Alpha-1-purothionin. |
| CHAIN    | 62   | 126 | 65     | Acidic protein.      |
| DISULFID | 19   | 55  |        |                      |
| DISULFID | 20   | 47  |        |                      |
| DISULFID | 28   | 45  |        |                      |
| DISULFID | 32   | 41  |        |                      |
| STRAND   | 18   | 20  | 3      |                      |
| HELIX    | 23   | 32  | 10     |                      |
| TURN     | 33   | 35  | 3      |                      |
| HELIX    | 38   | 45  | 8      |                      |
| TURN     | 46   | 46  | 1      |                      |
| STRAND   | 47   | 49  | 3      |                      |

**Sequence information**

Length: **126 AA** [This is the length of the partial sequence of the unprocessed precursor]      Molecular weight: **13526 Da** [This is the MW of the partial sequence of the unprocessed precursor]      CRC64: **FF7310D921C4EE30** [This is a checksum on the sequence]

```

      10      20      30      40      50      60
CLLILGLVLE QLQVEGKSCC RSTLGRNCYN LCRARGAQL CAGVCRCKIS SGLSCP KGF
      70      80      90     100     110     120
KLALESNSDE PDTIEYCNLG CRSSVCDYMV NAAADDEEMK LYVENCADAC VSFCNGDAGL

```

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ExPASy/SIB  
or at NCBI (USA)



Sequence analysis tools: ProtParam, ProtScale,  
Compute pI/Mw, PeptideMass, PeptideCutter,  
Dotlet (Java)



ScanProsite, MotifScan



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### Entry information

Entry name **THN21\_ARATH**  
 Primary accession number **Q42596**  
 Secondary accession numbers **Q8LCH6 Q9C7S9**  
 Entered in Swiss-Prot in **Release 43, March 2004**  
 Sequence was last modified in **Release 43, March 2004**  
 Annotations were last modified in **Release 46, February 2005**


### Name and origin of the protein

Protein name **Thionin 2.1 [Precursor]**  
 Synonyms **None**  
 Gene name **Name: THI2.1**  
**OrderedLocusNames: At1g72260**  
**ORFNames: T9N14.13**  
 From **Arabidopsis thaliana (Mouse-ear cress) [TaxID: 3702]**  
 Taxonomy **Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.**

### References

- [1] NUCLEOTIDE SEQUENCE, TISSUE SPECIFICITY, AND INDUCTION.  
**STRAIN**=cv. Columbia;  
 DOI=10.1104/pp.109.3.813;MEDLINE=96079536;PubMed=8552715 [NCBI, ExPASy, EBI, Israel, Japan]  
 Eppele P., Apel K., Bohlmann H.;  
 "An Arabidopsis thaliana thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins.";  
 Plant Physiol. 109:813-820(1995).
- [2] NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA].  
**STRAIN**=cv. Columbia;  
 DOI=10.1038/35048500;MEDLINE=21016719;PubMed=11130712 [NCBI, ExPASy, EBI, Israel,


Japan]

Theologis A., Ecker J.R., Palm C.J., Federspiel N.A., Kaul S., White O., Alonso J., Altafi H., Araujo R., Bowman C.L., Brooks S.Y., Buehler E., Chan A., Chao Q., Chen H., Cheuk R.F., Chin C.W., Chung M.K., Conn L., , Davis R.W.;  
"Sequence and analysis of chromosome 1 of the plant *Arabidopsis thaliana*.";  
Nature 408:816-820(2000).

[3] NUCLEOTIDE SEQUENCE [LARGE SCALE MRNA].

STRAIN=cv. Columbia;

DOI=10.1126/science.1088305;MEDLINE=22954850;PubMed=14593172 [NCBI, ExPASy, EBI, Israel, Japan]

Yamada K., Lim J., Dale J.M., Chen H., Shinn P., Palm C.J., Southwick A.M., Wu H.C., Kim C.J., Nguyen M., Pham P.K., Cheuk R.F., Karlin-Newmann G., Liu S.X., Lam B., Sakano H., Wu T., Yu G., Miranda M., , Ecker J.R.;  
"Empirical analysis of transcriptional activity in the *Arabidopsis* genome.";  
Science 302:842-846(2003).

[4] NUCLEOTIDE SEQUENCE [LARGE SCALE MRNA].

Brover V., Troukhan M., Alexandrov N., Lu Y.-P., Flavell R., Feldmann K.A.;  
"Full-length cDNA from *Arabidopsis thaliana*.";  
Submitted (MAR-2002) to the EMBL/GenBank/DDBJ databases.

[5] FUNCTION.

DOI=10.1105/tpc.9.4.509;PubMed=9144959 [NCBI, ExPASy, EBI, Israel, Japan]

Epple P., Apel K., Bohlmann H.;  
"Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*.";  
Plant Cell 9:509-520(1997).

[6] FUNCTION, TISSUE SPECIFICITY, AND INDUCTION.

PubMed=9628023 [NCBI, ExPASy, EBI, Israel, Japan]  
Vignutelli A., Wasternack C., Apel K., Bohlmann H.;  
"Systemic and local induction of an *Arabidopsis* thionin gene by wounding and pathogens.";  
Plant J. 14:285-295(1998).

Comments

- **FUNCTION:** Seems to function as a defense factor. Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- **SUBCELLULAR LOCATION:** Secreted (*Potential*).
- **TISSUE SPECIFICITY:** Detected in rosette leaves and at a very high level in flowers and in siliques.
- **INDUCTION:** Highly induced in seedlings by pathogens, wounding, silver nitrate, and methyl jasmonate.
- **SIMILARITY:** Belongs to the plant thionin (TC 1.C.44) family [view classification].

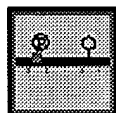
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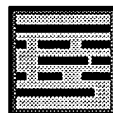
Cross-references

|      |                                 |                                             |
|------|---------------------------------|---------------------------------------------|
|      | L41244; AAC41678.1; -.          | [EMBL / GenBank / DDBJ]<br>[CoDingSequence] |
| EMBL | AC067754; AAG51790.1; ALT_INIT. | [EMBL / GenBank / DDBJ]<br>[CoDingSequence] |
|      | AY080781; AAL87264.1; -.        | [EMBL / GenBank / DDBJ]                     |

[CoDingSequence]  
 [EMBL / GenBank / DDBJ]  
 [CoDingSequence]  
 AY086595; AAM63655.1;  
 ALT\_INIT.  
 PIR C96746; C96746.  
 HSSP P32880; 1JMN. [HSSP ENTRY / PDB]  
 GeneFarm 3048; 293.  
 TAIR At1g72260; Q42596.  
 InterPro IPR001010; Thionin.  
 Graphical view of domain structure.  
 Pfam PF00321; Thionin; 1.  
 Pfam graphical view of domain structure.  
 PROSITE PS00271; THIONIN; 1.  
 ProDom [Domain structure / List of seq. sharing at least 1 domain]  
 BLOCKS Q42596.  
 ProtoNet Q42596.  
 ProtoMap Q42596.  
 PRESAGE Q42596.  
 DIP Q42596.  
 ModBase Q42596.  
 SMR Q42596; 515B40A0DBDCC1EF.  
 SWISS-2DPAGE Get region on 2D PAGE.  
 UniRef View cluster of proteins with at least 50% / 90% identity.

**Keywords****Plant defense; Plant toxin; Signal; Thionin; Toxin.****Features**

Feature table viewer



Feature aligner

| Key      | From | To  | Length | Description                 |
|----------|------|-----|--------|-----------------------------|
| SIGNAL   | 1    | 24  | 24     | Potential.                  |
| CHAIN    | 25   | 67  | 43     | Thionin 2.1.                |
| CHAIN    | 68   | 134 | 67     | Acidic protein (Potential). |
| DISULFID | 27   | 61  |        | By similarity.              |
| DISULFID | 28   | 55  |        | By similarity.              |
| DISULFID | 40   | 49  |        | By similarity.              |
| CONFLICT | 7    | 7   |        | I -> S (in Ref. 4).         |
| CONFLICT | 74   | 74  |        | A -> G (in Ref. 4).         |

**Sequence information**

Length: 134 AA [This is the length of the unprocessed precursor]  
 Molecular weight: 14351 Da [This is the MW of the unprocessed precursor]

CRC64: 515B40A0DBDCC1EF [This is a checksum on the sequence]

```

      10      20      30      40      50      60
MKGRILILSL LIMSLVMAQV QVEAKICCP S NQARNGYSVC RIRFSKGRCM QVSGCQNSDT
      70      80      90     100     110     120
  
```

CPRGWVNAIL ENSADATNEH CKLGCETSVG GAMNTLQNSD ASEIVNGASE QCAKGCSIFC

130



TKSYVVPFPG PKLL

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or at NCBI (USA)Sequence analysis tools: ProtParam, ProtScale,  
Compute pI/Mw, PeptideMass, PeptideCutter,  
Dotlet (Java)

ScanProsite, MotifScan



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### Entry information

|                                   |                           |
|-----------------------------------|---------------------------|
| Entry name                        | <b>THN2_WHEAT</b>         |
| Primary accession number          | <b>P32032</b>             |
| Secondary accession numbers       | None                      |
| Entered in Swiss-Prot in          | Release 26, July 1993     |
| Sequence was last modified in     | Release 26, July 1993     |
| Annotations were last modified in | Release 46, February 2005 |

### Name and origin of the protein

|              |                                                                                                                                                                    |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Protein name | <b>Alpha-2-purothionin [Precursor]</b>                                                                                                                             |
| Synonyms     | None                                                                                                                                                               |
| Gene name    | <b>Name: THI1.2</b><br>Synonyms: PUR-B1                                                                                                                            |
| From         | Triticum aestivum (Wheat) [TaxID: 4565]                                                                                                                            |
| Taxonomy     | Eukaryota; Viridiplantae; Streptophyta; Embryophyta;<br>Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales;<br>Poaceae; Pooideae; Triticeae; Triticum. |

### References

#### [1] NUCLEOTIDE SEQUENCE.

**TISSUE**=Endosperm;

DOI=10.1104/pp.106.3.1221;MEDLINE=95125120;PubMed=7824649 [NCBI, ExPASy, EBI, Israel, Japan]

Castagnaro A., Marana C., Carbonero P., Garcia-Olmedo F.;

"cDNA cloning and nucleotide sequences of alpha 1 and alpha 2 thionins from hexaploid wheat endosperm.";

Plant Physiol. 106:1221-1222(1994).

### Comments

- **FUNCTION:** Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- **SUBCELLULAR LOCATION:** Secreted.
- **SIMILARITY:** Belongs to the plant thionin (TC 1.C.44) family [view classification].

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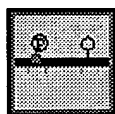
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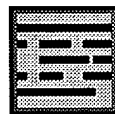
|              |                                                                  |
|--------------|------------------------------------------------------------------|
| EMBL         | X70665; CAA50003.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]   |
| PIR          | S31695; S31695.                                                  |
| HSSP         | P01543; 1BHP. [HSSP ENTRY / PDB]                                 |
| InterPro     | IPR001010; Thionin.<br>Graphical view of domain structure.       |
| Pfam         | PF00321; Thionin; 1.<br>Pfam graphical view of domain structure. |
| PRINTS       | PR00287; THIONIN.                                                |
| PROSITE      | PS00271; THIONIN; 1.                                             |
| ProDom       | [Domain structure / List of seq. sharing at least 1 domain]      |
| BLOCKS       | P32032.                                                          |
| ProtoNet     | P32032.                                                          |
| ProtoMap     | P32032.                                                          |
| PRESAGE      | P32032.                                                          |
| DIP          | P32032.                                                          |
| ModBase      | P32032.                                                          |
| SMR          | P32032; B4019F014E226B9F.                                        |
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| UniRef       | View cluster of proteins with at least 50% / 90% identity.       |

**Keywords**

**Plant defense; Plant toxin; Signal; Thionin.**

**Features**

Feature table viewer



Feature aligner

| Key      | From | To  | Length | Description          |
|----------|------|-----|--------|----------------------|
| SIGNAL   | 1    | 27  | 27     |                      |
| CHAIN    | 28   | 72  | 45     | Alpha-2-purothionin. |
| CHAIN    | 73   | 136 | 64     | Acidic protein.      |
| DISULFID | 30   | 66  |        | By similarity.       |
| DISULFID | 31   | 58  |        | By similarity.       |
| DISULFID | 39   | 56  |        | By similarity.       |
| DISULFID | 43   | 52  |        | By similarity.       |

**Sequence information**

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14558 Da** [This is the MW of the unprocessed precursor]

CRC64: **B4019F014E226B9F** [This is a checksum on the sequence]

```

      10      20      30      40      50      60
MGSKGLKGV M VCLLILGLVL EQVQVEGKSC CRTTLGRNCY NLCSRGAQK LCSTVCRCKL
      70      80      90     100     110     120

```

TSGLSCPKGF PKLALESNSD EPDTIEYCNL GCRSSVCDYM VNAAADDEEM KLYVENCGDA

130  
CVNFCNGDAG LTSLDA

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ExPASy/SIB  
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Sequence analysis tools: ProtParam, ProtScale,  
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### Entry information

|                                   |                           |
|-----------------------------------|---------------------------|
| Entry name                        | <b>THNB_WHEAT</b>         |
| Primary accession number          | <b>P01543</b>             |
| Secondary accession numbers       | None                      |
| Entered in Swiss-Prot in          | Release 01, July 1986     |
| Sequence was last modified in     | Release 36, July 1998     |
| Annotations were last modified in | Release 46, February 2005 |

### Name and origin of the protein

|              |                                                                                                                                                                    |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Protein name | <b>Purothionin A-I [Precursor]</b>                                                                                                                                 |
| Synonym      | <b>Beta-purothionin</b>                                                                                                                                            |
| Gene name    | <b>Name: THI1.3</b>                                                                                                                                                |
| From         | Triticum aestivum (Wheat) [TaxID: 4565]                                                                                                                            |
| Taxonomy     | Eukaryota; Viridiplantae; Streptophyta; Embryophyta;<br>Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales;<br>Poaceae; Pooideae; Triticeae; Triticum. |

### References

#### [1] NUCLEOTIDE SEQUENCE.

**STRAIN**=cv. Rosella;  
Hughes P.A., Llewellyn D.L., Whitecross M.;  
Submitted (JUN-1997) to the EMBL/GenBank/DDBJ databases.

#### [2] PROTEIN SEQUENCE OF 28-72.

**STRAIN**=cv. Manitoba 3;  
**MEDLINE**=78026451; **PubMed**=914810 [NCBI, ExPASy, EBI, Israel, Japan]  
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;  
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast from wheat flour."  
J. Biochem. 82:753-767(1977).

#### [3] PROTEIN SEQUENCE OF 28-72.

Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;  
"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";

Agric. Biol. Chem. 39:2269-2270(1975).

[4] PROTEIN SEQUENCE OF 28-72.

MEDLINE=77046666;PubMed=990986 [NCBI, ExPASy, EBI, Israel, Japan]

Mak A.S., Jones B.L.;

"The amino acid sequence of wheat beta-purothionin.";

Can. J. Biochem. 54:835-842(1976).

[5] X-RAY CRYSTALLOGRAPHY (1.7 ANGSTROMS).

DOI=10.1107/S0907444995002976;PubMed=15299761 [NCBI, ExPASy, EBI, Israel, Japan]

Stec B., Rao U., Teeter M.M.;

"Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: structure of beta-purothionin at 1.7-A resolution.";

Acta Crystallogr. D 51:914-924(1995).

**Comments**

- **FUNCTION:** Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- **SUBCELLULAR LOCATION:** Secreted.
- **SIMILARITY:** Belongs to the plant thionin (TC 1.C.44) family [view classification].

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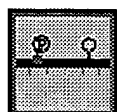
**Cross-references**

|              |                                                                   |
|--------------|-------------------------------------------------------------------|
| EMBL         | AF004018; AAB71137.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] |
| PDB          | 1BHP; X-ray; @=28-71. [ExPASy / RCSB / EBI]                       |
| InterPro     | IPR001010; Thionin.<br>Graphical view of domain structure.        |
| Pfam         | PF00321; Thionin; 1.<br>Pfam graphical view of domain structure.  |
| PRINTS       | PR00287; THIONIN.                                                 |
| PROSITE      | PS00271; THIONIN; 1.                                              |
| ProDom       | [Domain structure / List of seq. sharing at least 1 domain]       |
| BLOCKS       | P01543.                                                           |
| ProtoNet     | P01543.                                                           |
| ProtoMap     | P01543.                                                           |
| PRESAGE      | P01543.                                                           |
| DIP          | P01543.                                                           |
| ModBase      | P01543.                                                           |
| SMR          | P01543; A855C815519EDA24.                                         |
| SWISS-2DPAGE | Get region on 2D PAGE.                                            |
| UniRef       | View cluster of proteins with at least 50% / 90% identity.        |

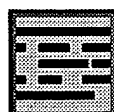
**Keywords**

**3D-structure; Direct protein sequencing; Plant defense; Plant toxin; Signal; Thionin.**

**Features**



Feature table viewer



Feature aligner

| Key      | From | To  | Length | Description      |
|----------|------|-----|--------|------------------|
| SIGNAL   | 1    | 27  | 27     |                  |
| CHAIN    | 28   | 72  | 45     | Purothionin A-I. |
| CHAIN    | 73   | 136 | 64     | Acidic protein.  |
| DISULFID | 30   | 66  |        |                  |
| DISULFID | 31   | 58  |        |                  |
| DISULFID | 39   | 56  |        |                  |
| DISULFID | 43   | 52  |        |                  |
| STRAND   | 29   | 31  | 3      |                  |
| HELIX    | 34   | 43  | 10     |                  |
| TURN     | 44   | 46  | 3      |                  |
| HELIX    | 49   | 55  | 7      |                  |
| TURN     | 56   | 57  | 2      |                  |
| STRAND   | 58   | 60  | 3      |                  |
| TURN     | 68   | 69  | 2      |                  |

**Sequence information**

Length: **136 AA** [This is the length of the unprocessed precursor]      Molecular weight: **14625 Da** [This is the MW of the unprocessed precursor]      CRC64: **A855C815519EDA24** [This is a checksum on the sequence]

```

      10      20      30      40      50      60
MGSKGLKGVM VCLLILGLVL EQVQVEGKSC CKSTLGRNCY NLCRARGAQK LCVNCRCKL

      70      80      90     100     110     120
TSGLSCP KDF PKLVLESNSD EPDTMEYCNL GCRSSLCDYI VNAAADDEEM KLYVEQCGDA

     130
CVNFCNADAG L TSLDA

```

P01543 in FASTA  
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Dotlet (Java)



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Acta Crystallographica Section D

# Biological Crystallography

Volume 51, Part 6 (November 1995)

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## research papers


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[pdf](#) [cited in](#) [similar papers](#) [contents of issue](#) [buy](#)*Acta Cryst.* (1995). D51, 914-924 [doi:10.1107/S0907444995002976]

### Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: structure of $\beta$ -purothionin at 1.7 Å resolution

**B. Stec, U. Rao and M. M. Teeter**

**Abstract:** The crystal structure of  $\beta$ -purothionin ( $\beta$ -PT) has been determined at 1.7 Å resolution.  $\beta$ -PT and previously solved  $\alpha_1$ -PT belong to a family of membrane-active plant toxins homologous to crambin. ( $\beta$ -PT crystallizes in the same space group as  $\alpha_1$ -PT (1422) but with the  $c$  axis 3 Å longer than ( $\alpha_1$ -PT. The unit-cell dimensions of  $\beta$ -PT crystals are  $a = b = 53.94$  and  $c = 72.75$  Å. Two data sets were collected on a multiwire area detector, each with  $R_{\text{sym}}$  around 6.0%, and were merged to get a single data set at 1.7 Å, ( $R_{\text{merge}} = 9.6\%$ ). The X-ray structure of  $\alpha_1$ -PT was used to build a starting model for  $\beta$ -PT. The  $\beta$ -PT model was refined using the program *PROLSQ* from 10 to 1.7 Å resolution to an  $R$ -factor of 19.8% with very good geometry. The final structure contains 439 atoms including 337 protein atoms, 77 waters, two acetates, two glycerols and one phosphate. The high-resolution structure of the  $\beta$ -PT agreed well with that of the lower resolution  $\alpha_1$ -PT structure only after the latter was extensively rerefined. Both refinements revealed phosphate and glycerol molecules which are important in lattice formation. The binding of phosphate and glycerol molecules to purothionins (PT) was confirmed by NMR and was implicated in the biological activity of toxins. Modeling of phospholipid binding to PT based on glycerol and phosphate-binding site could shed light on the lytic toxicity of this protein-toxin family. Although the structures of ( $\alpha_1$ -PT and  $\beta$ -PT preserve the overall fold of crambin, they exhibit key differences that are directly relevant to the toxicity of thionins.

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If your question is not covered, please contact [<helpdesk@expasy.org>](mailto:helpdesk@expasy.org).

NCBI BLAST program reference [PMID:9254694]:

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402(1997).

=====

Query: 136 AA

Date run: 2005-02-24 18:07:40 UTC+0100 on sib-gml.unil.ch

Program: NCBI BLASTP 1.5.4-Paracel [2003-06-05]

Database: EXPASY/UniProt

1,794,555 sequences; 574,459,479 total letters

UniProt Release 4.1 consists of: Swiss-Prot Release 46.1 of 15-Feb-2005: 170140 ent

TrEMBL Release 29.1 of 15-Feb-2005: 1614107 entries

#### List of potentially matching sequences

Send selected sequences to 

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|                          | Db | AC     | Description                                                 | Score | E-value |
|--------------------------|----|--------|-------------------------------------------------------------|-------|---------|
| <input type="checkbox"/> | sp | P32032 | THN2_WHEAT Alpha-2-purothionin precursor [THI1.2] [Tri...   | 288   | 1e-77   |
| <input type="checkbox"/> | tr | Q43205 | _WHEAT Alpha-1 purothionin [Triticum aestivum (Wheat)]      | 285   | 8e-77   |
| <input type="checkbox"/> | sp | P01543 | THNB_WHEAT Purothionin A-I precursor (Beta-purothionin...   | 268   | 1e-71   |
| <input type="checkbox"/> | tr | Q9T0P1 | _WHEAT Alpha purothionin precursor [Pur-B1] [Triticum a...  | 268   | 1e-71   |
| <input type="checkbox"/> | tr | Q9T0P2 | _WHEAT Beta purothionin precursor [Pur-A1] [Triticum ae...  | 267   | 3e-71   |
| <input type="checkbox"/> | sp | P21742 | THNB_HORVU Beta-hordothionin precursor [THI1.2] [Horde...   | 257   | 2e-68   |
| <input type="checkbox"/> | tr | Q9ZNY5 | _SECCE Purothionin precursor [Pur-RL] [Secale cereale (...] | 257   | 3e-68   |
| <input type="checkbox"/> | sp | P01544 | THN1_WHEAT Alpha-1-purothionin precursor (Purothionin ...   | 251   | 2e-66   |

|                          |    |                        |                                                            |                     |       |
|--------------------------|----|------------------------|------------------------------------------------------------|---------------------|-------|
| <input type="checkbox"/> | sp | <a href="#">P01545</a> | THNA_HORVU Alpha-hordothionin precursor (Purothionin I...  | <a href="#">243</a> | 5e-64 |
| <input type="checkbox"/> | tr | <a href="#">Q9S6Y2</a> | _WHEAT Alpha purothionin (Fragment) [Pur-B1] [Triticum ... | <a href="#">181</a> | 2e-45 |
| <input type="checkbox"/> | tr | <a href="#">Q9S9D7</a> | _HORVU Thionin [Hordeum vulgare (Barley)]                  | <a href="#">155</a> | 2e-37 |
| <input type="checkbox"/> | sp | <a href="#">Q42838</a> | THN7_HORVU Thionin BTH7 precursor [Hordeum vulgare (Ba...  | <a href="#">154</a> | 3e-37 |
| <input type="checkbox"/> | tr | <a href="#">Q8LT03</a> | _AVESA Leaf thionin Asthi1 [Asthi1] [Avena sativa (Oat)]   | <a href="#">154</a> | 4e-37 |
| <input type="checkbox"/> | sp | <a href="#">P08772</a> | THN3_HORVU Leaf-specific thionin DB4 precursor [THI1.3...  | <a href="#">153</a> | 6e-37 |
| <input type="checkbox"/> | sp | <a href="#">Q8H0Q5</a> | THNX_HORVU Probable leaf thionin precursor [Hordeum vu...  | <a href="#">152</a> | 1e-36 |
| <input type="checkbox"/> | sp | <a href="#">P09617</a> | THN5_HORVU Leaf-specific thionin precursor (Clones PKG...  | <a href="#">152</a> | 1e-36 |
| <input type="checkbox"/> | sp | <a href="#">P09618</a> | THN6_HORVU Leaf-specific thionin BTH6 precursor [Horde...  | <a href="#">149</a> | 9e-36 |
| <input type="checkbox"/> | tr | <a href="#">Q8LT02</a> | _AVESA Leaf thionin Asthi2 [Asthi2] [Avena sativa (Oat)]   | <a href="#">145</a> | 1e-34 |
| <input type="checkbox"/> | tr | <a href="#">Q8LSZ9</a> | _AVESA Thionin Asthi5 [Asthi5] [Avena sativa (Oat)]        | <a href="#">138</a> | 2e-32 |
| <input type="checkbox"/> | tr | <a href="#">Q8LT00</a> | _AVESA Thionin Asthi4 [Asthi4] [Avena sativa (Oat)]        | <a href="#">134</a> | 2e-31 |
| <input type="checkbox"/> | tr | <a href="#">Q41585</a> | _WHEAT Type V Thionin precursor [TthV2] [Triticum aesti... | <a href="#">134</a> | 4e-31 |
| <input type="checkbox"/> | sp | <a href="#">Q05806</a> | THN5_WHEAT Type V thionin precursor [TTHV] [Triticum a...  | <a href="#">130</a> | 6e-30 |
| <input type="checkbox"/> | tr | <a href="#">Q8LT01</a> | _AVESA Leaf thionin Asthi3 [Asthi3] [Avena sativa (Oat)]   | <a href="#">129</a> | 8e-30 |
| <input type="checkbox"/> | tr | <a href="#">Q38770</a> | _AEGTA Type V Thionin precursor [AthV1] [Aegilops tausc... | <a href="#">129</a> | 8e-30 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z540</a> | _ORYSA Putative thionin Osthi1 [OSJNBa0085C03.54] [Oryz... | <a href="#">111</a> | 2e-24 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z4W6</a> | _ORYSA Putative thionin Osthi1 [OSJNBb0071G09.18] [Oryz... | <a href="#">108</a> | 2e-23 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z5V1</a> | _ORYSA Putative thionin Osthi1 [OSJNBa0020P04.23] [Oryz... | <a href="#">106</a> | 9e-23 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z434</a> | _ORYSA Putative thionin Osthi1 [OSJNBa0061G23.50] [Oryz... | <a href="#">105</a> | 2e-22 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z4S4</a> | _ORYSA Putative thionin Osthi1 [P0597A07.39] [Oryza sat... | <a href="#">102</a> | 1e-21 |
| <input type="checkbox"/> | tr | <a href="#">Q8LT04</a> | _ORYSA Thionin Osthi1 [Osthi1] [Oryza sativa (japonica ... | <a href="#">102</a> | 1e-21 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z4K0</a> | _ORYSA Thionin Osthi1 [OSJNBa0022O06.36] [Oryza sativa ... | <a href="#">101</a> | 2e-21 |
| <input type="checkbox"/> | tr | <a href="#">Q9S975</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA11 (Frag... | <a href="#">90</a>  | 7e-18 |
| <input type="checkbox"/> | tr | <a href="#">Q43225</a> | _TULGE Thionin class 1 precursor [Thi1-2] [Tulipa gesne... | <a href="#">90</a>  | 7e-18 |
| <input type="checkbox"/> | sp | <a href="#">Q9SBK8</a> | THN_BRARP Thionin precursor [THI2] [Brassica rapa subs...  | <a href="#">89</a>  | 1e-17 |
| <input type="checkbox"/> | tr | <a href="#">Q43224</a> | _TULGE Thionin class 1 precursor [Thi1-1] [Tulipa gesne... | <a href="#">88</a>  | 3e-17 |
| <input type="checkbox"/> | tr | <a href="#">Q9S976</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA10 (Frag... | <a href="#">86</a>  | 1e-16 |
| <input type="checkbox"/> | tr | <a href="#">Q43226</a> | _TULGE Thionin class 1 precursor [Thi1-3] [Tulipa gesne... | <a href="#">86</a>  | 1e-16 |
| <input type="checkbox"/> | tr | <a href="#">Q9S981</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA2 (Fragm... | <a href="#">85</a>  | 2e-16 |
| <input type="checkbox"/> | tr | <a href="#">Q9S974</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA12 (Frag... | <a href="#">84</a>  | 4e-16 |
| <input type="checkbox"/> | tr | <a href="#">Q9S9A0</a> | _VISAL Thionin [Viscum album (European mistletoe)]         | <a href="#">83</a>  | 8e-16 |
| <input type="checkbox"/> | sp | <a href="#">Q42597</a> | THN22_ARATH Thionin 2.2 precursor [THI2.2] [Arabidopsi...  | <a href="#">83</a>  | 1e-15 |
| <input type="checkbox"/> | tr | <a href="#">Q43227</a> | _TULGE Thionin class 1 precursor (Fragment) [Thi1-4] [T... | <a href="#">82</a>  | 2e-15 |
| <input type="checkbox"/> | tr | <a href="#">Q9S980</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA3 (Fragm... | <a href="#">81</a>  | 4e-15 |
| <input type="checkbox"/> | tr | <a href="#">Q9S977</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA9 (Fragm... | <a href="#">80</a>  | 5e-15 |
| <input type="checkbox"/> | sp | <a href="#">Q42596</a> | THN21_ARATH Thionin 2.1 precursor [THI2.1] [Arabidopsi...  | <a href="#">79</a>  | 1e-14 |
| <input type="checkbox"/> | sp | <a href="#">P01538</a> | THN3_VISAL Viscotoxin A3 precursor [THI2.1] [Viscum al...  | <a href="#">77</a>  | 4e-14 |
| <input type="checkbox"/> | tr | <a href="#">Q9S9A2</a> | _VISAL Viscotoxin A [Viscum album (European mistletoe)]    | <a href="#">77</a>  | 4e-14 |
| <input type="checkbox"/> | tr | <a href="#">Q9S9A1</a> | _VISAL Thionin [Viscum album (European mistletoe)]         | <a href="#">74</a>  | 6e-13 |
| <input type="checkbox"/> | tr | <a href="#">Q9S979</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA5 (Fragm... | <a href="#">73</a>  | 8e-13 |
| <input type="checkbox"/> | sp | <a href="#">P08943</a> | THNB_VISAL Viscotoxin B precursor (Fragment) [THI2.2] ...  | <a href="#">72</a>  | 1e-12 |
| <input type="checkbox"/> | sp | <a href="#">Q8VZK8</a> | THN23_ARATH Probable thionin 2.3 precursor [At2g15010]...  | <a href="#">72</a>  | 2e-12 |
| <input type="checkbox"/> | tr | <a href="#">Q41609</a> | _TULGE Thionin class 4 precursor [Thi4-1] [Tulipa gesne... | <a href="#">72</a>  | 2e-12 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z554</a> | _ORYSA Putative thionin Osthi1 [OSJNBa0085C03.20] [Oryz... | <a href="#">71</a>  | 3e-12 |

|                          |    |                        |                                                            |                    |       |
|--------------------------|----|------------------------|------------------------------------------------------------|--------------------|-------|
| <input type="checkbox"/> | sp | <a href="#">Q9C8D6</a> | THN24_ARATH Probable thionin 2.4 precursor [At1g66100]...  | <a href="#">65</a> | 2e-10 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z551</a> | _ORYSA Putative thionin Osth1 [OSJNBa0085C03.23] [Oryz...  | <a href="#">61</a> | 3e-09 |
| <input type="checkbox"/> | sp | <a href="#">P07504</a> | THN_PYRPU Thionin [THI1] [Pyrularia pubera (Rabbitwood...  | <a href="#">61</a> | 4e-09 |
| <input type="checkbox"/> | sp | <a href="#">P60057</a> | THND_HELPU Hellethionin D [Helleborus purpurascens (Pu...  | <a href="#">52</a> | 2e-06 |
| <input type="checkbox"/> | sp | <a href="#">P83554</a> | THNC_VISAL Viscotoxin C1 [Viscum album (European mistl...  | <a href="#">51</a> | 4e-06 |
| <input type="checkbox"/> | sp | <a href="#">P01541</a> | THN_DENCL Denclatoxin B [Dendrophthora clavata (Columb...  | <a href="#">50</a> | 6e-06 |
| <input type="checkbox"/> | sp | <a href="#">P59358</a> | THNB_PHOLI Ligatoxin B [Phoradendron liga (Argentine m...  | <a href="#">48</a> | 4e-05 |
| <input type="checkbox"/> | sp | <a href="#">P01539</a> | THN_PHOTO Phoratoxin [Phoradendron tomentosum (Califor...  | <a href="#">47</a> | 6e-05 |
| <input type="checkbox"/> | sp | <a href="#">P32880</a> | THN2_VISAL Viscotoxin A2 [THI2.3] [Viscum album (Europ...  | <a href="#">47</a> | 6e-05 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z553</a> | _ORYSA Hypothetical protein OSJNBa0085C03.21 [OSJNBa008... | <a href="#">47</a> | 8e-05 |
| <input type="checkbox"/> | sp | <a href="#">P01540</a> | THNA_PHOLI Ligatoxin A [Phoradendron liga (Argentine m...  | <a href="#">45</a> | 2e-04 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z4S0</a> | _ORYSA Thionin Osth1-like [P0597A07.48] [Oryza sativa ...  | <a href="#">45</a> | 2e-04 |
| <input type="checkbox"/> | sp | <a href="#">P01537</a> | THN1_VISAL Viscotoxin 1-PS [THI2.4] [Viscum album (Eur...  | <a href="#">44</a> | 4e-04 |
| <input type="checkbox"/> | tr | <a href="#">Q5Z557</a> | _ORYSA Hypothetical protein OSJNBa0085C03.15 [OSJNBa008... | <a href="#">44</a> | 4e-04 |
| <input type="checkbox"/> | tr | <a href="#">Q9S978</a> | _CRAAB Crambin PRECURSOR=THIONIN variant THI2CA7 (Fragm... | <a href="#">42</a> | 0.002 |
| <input type="checkbox"/> | sp | <a href="#">Q88745</a> | SCRG1_MOUSE Scrapie-responsive protein 1 precursor (Sc...  | <a href="#">35</a> | 0.19  |
| <input type="checkbox"/> | sp | <a href="#">P01542</a> | CRAM_CRAAB Crambin [THI2] [Crambe abyssinica (Abyssini...  | <a href="#">35</a> | 0.25  |
| <input type="checkbox"/> | sp | <a href="#">Q9Z0K6</a> | SCRG1_RAT Scrapie-responsive protein 1 precursor (ScRG...  | <a href="#">34</a> | 0.43  |
| <input type="checkbox"/> | tr | <a href="#">Q9LN81</a> | _ARATH T12C24.19 [Arabidopsis thaliana (Mouse-ear cress)]  | <a href="#">34</a> | 0.57  |
| <input type="checkbox"/> | tr | <a href="#">Q7Q1J5</a> | _ANOCA ENSANGP00000014375 (Fragment) [ENSANGG0000001188... | <a href="#">33</a> | 0.74  |
| <input type="checkbox"/> | tr | <a href="#">Q8W2K6</a> | _9SOLN Proteinase inhibitor IIa [PIN2a] [Solanum americ... | <a href="#">33</a> | 0.96  |
| <input type="checkbox"/> | tr | <a href="#">Q8MY77</a> | _BRABE Bb-cadherin [BbCad] [Branchiostoma belcheri (Amp... | <a href="#">32</a> | 1.6   |
| <input type="checkbox"/> | tr | <a href="#">Q8BKK7</a> | _MOUSE Mus musculus 12 days embryo spinal ganglion cDNA... | <a href="#">32</a> | 2.1   |
| <input type="checkbox"/> | tr | <a href="#">Q95V69</a> | _TETTH Cell surface immobilization antigen SerH6 [SerH]... | <a href="#">32</a> | 2.8   |
| <input type="checkbox"/> | tr | <a href="#">Q60XC0</a> | _CAEBR Hypothetical protein CBG18730 (Fragment) [CBG187... | <a href="#">32</a> | 2.8   |
| <input type="checkbox"/> | sp | <a href="#">P04881</a> | NCAP_VSVJO Nucleocapsid protein (Nucleoprotein) [N] [V...  | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89779</a> | _9RHAB (strain 09/82-HD-B) nucleoprotein ((strain 06/85... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89594</a> | _9RHAB (strain 10/85-HD-B1) nucleoprotein ((strain 12/8... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89520</a> | _9RHAB (strain ../49-UT-B1) nucleoprotein ((strain ../5... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89040</a> | _9RHAB (strain ../60-PN-B) nucleoprotein [Vesicular sto... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89039</a> | _9RHAB (strain 01/85-PN-B1) nucleoprotein [Vesicular st... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89038</a> | _9RHAB (strain 07/83-NC-P) nucleoprotein [Vesicular sto... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89037</a> | _9RHAB (strain 10/82-CR-B) nucleoprotein [Vesicular sto... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89036</a> | _9RHAB (strain 11/84-HD-B1) nucleoprotein [Vesicular st... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89035</a> | _9RHAB (strain 10/84-GM-P) nucleoprotein [Vesicular sto... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q89034</a> | _9RHAB (strain 07/84-OA-B) nucleoprotein [Vesicular sto... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q6TXD9</a> | _9RHAB Nucleocapsid protein [Vesicular stomatitis virus]   | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q9CA14</a> | _ARATH Putative phorbol ester / diacylglycerol binding ... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q620M9</a> | _CAEBR Hypothetical protein CBG02727 [CBG02727] [Caenor... | <a href="#">31</a> | 3.7   |
| <input type="checkbox"/> | tr | <a href="#">Q84BD4</a> | _MYXXA CHP [Myxococcus xanthus]                            | <a href="#">31</a> | 4.8   |
| <input type="checkbox"/> | tr | <a href="#">Q69JY6</a> | _ORYSA Putative thaumatin-like protein [P0569E11.21] [O... | <a href="#">31</a> | 4.8   |
| <input type="checkbox"/> | tr | <a href="#">Q6PTE0</a> | _MONBE Methionine adenosyltransferase (Fragment) [Monos... | <a href="#">31</a> | 4.8   |
| <input type="checkbox"/> | tr | <a href="#">Q6PVY8</a> | _ORYSA Zinc finger protein [Tranpr] [Oryza sativa (japo... | <a href="#">30</a> | 6.3   |
| <input type="checkbox"/> | tr | <a href="#">Q6AVT3</a> | _ORYSA Hypothetical protein OSJNBa0027J18.18 [OSJNBa002... | <a href="#">30</a> | 6.3   |
| <input type="checkbox"/> | tr | <a href="#">Q95RQ1</a> | _DROME LD16414p (CG7447-PA, isoform A) (Cg7447-pb, isof... | <a href="#">30</a> | 6.3   |

☐ tr Q7PM75 \_ANOGA ENSANGP00000010141 (Fragment) [ENSANGG00000000765... 30 6.3  
☐ sp P79847 LYSC\_PYGNE Lysozyme C precursor (EC 3.2.1.17) (1,4-bet... 30 8.2

**Graphical overview of the alignments**[Click here](#)

to resubmit your query after masking regions matching PROSITE profiles  
or Pfam HMMs

([?](#) Help) (use ScanProsite for more details about PROSITE matches)

**Profile hits****Pfan hits****Thionin**



| Submission  | Matches on query sequence |  |  |  |  |  |  |  |  |  | Mat   |
|-------------|---------------------------|--|--|--|--|--|--|--|--|--|-------|
|             | 1                         |  |  |  |  |  |  |  |  |  | 1     |
| THN2_WHEAT  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q43205      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNB_WHEAT  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9T0P1      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9T0P2      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNB_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9ZNY5      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN1_WHEAT  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNA_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S6Y2      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S9D7      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN7_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LT03      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN3_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNX_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN5_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN6_HORVU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LT02      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LSZ9      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LT00      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q41585      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN5_WHEAT  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LT01      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q38770      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z540      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z4M6      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z5V1      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z434      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z454      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8LT04      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z4K0      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S975      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q43225      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN_BRARP   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q43224      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S976      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q43226      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S981      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S974      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S9A0      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN22_ARATH | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q43227      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S980      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S977      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN21_ARATH | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN3_VISAL  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S9A2      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S9A1      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S979      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNB_VISAL  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN23_ARATH | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q41609      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z554      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN24_ARATH | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z551      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN_PYRPU   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THND_HELPU  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNC_VISAL  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN_DENCL   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNB_PHOLI  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN_PHOTO   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN2_VISAL  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z553      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THNA_PHOLI  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z450      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| THN1_VISAL  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q5Z557      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S978      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| SCRG1_MOUSE | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| CRAM_CRAB   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| SCRG1_RAT   | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9LN81      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q7Q1J5      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8M2K6      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8MY77      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q8BK7       | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q9S969      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q60XC0      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| NCAP_VSVJO  | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89779      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89594      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89520      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89040      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89039      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89038      | =====                     |  |  |  |  |  |  |  |  |  | ===== |
| Q89037      | =====                     |  |  |  |  |  |  |  |  |  | ===== |

## Alignments

sp P32032 **Alpha-2-purothionin precursor [THI1.2] [Triticum aestivum** 136  
THN2\_WHEAT **(Wheat)]** AA  
align

Score = 288 bits (737), Expect = 1e-77  
Identities = 136/136 (100%), Positives = 136/136 (100%)

Query: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL 60  
MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL  
Sbjct: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

Query: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120  
TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA  
Sbjct: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120

Query: 121 CVNFCNGDAGLTS LDA 136  
CVNFCNGDAGLTS LDA  
Sbjct: 121 CVNFCNGDAGLTS LDA 136

tr Q43205 **Alpha-1 purothionin [Triticum aestivum** 136 AA  
Q43205\_WHEAT **(Wheat)]** align

Score = 285 bits (730), Expect = 8e-77  
Identities = 135/136 (99%), Positives = 135/136 (99%)

Query: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL 60  
MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL  
Sbjct: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

Query: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120  
TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA  
Sbjct: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120

Query: 121 CVNFCNGDAGLTS LDA 136  
CVNFCNGDAGLTS DA  
Sbjct: 121 CVNFCNGDAGLTS LDA 136

sp P01543 **Purothionin A-I precursor (Beta-purothionin) [THI1.3]** 136  
THNB\_WHEAT **[Triticum** AA  
**aestivum (Wheat)]** align

Score = 268 bits (686), Expect = 1e-71  
Identities = 124/136 (91%), Positives = 131/136 (96%)

Query: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKL 60  
MGSKGLKGMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+ VCRCKL  
Sbjct: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRARGAQKLCANVCRCKL 60

Query: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120

Sbjct: 61 TSGLSCPKEFKLVLESNSDEPDTMEYCNLGCRSSLCDYIVNAAADDEEMKLYVEQCGDA 120

Query: 121 CVNFCNGDAGLTSlda 136  
CVNFCN DAGLTSlda

Sbjct: 121 CVNFCNADAGLTSlda 136

tr Q9T0P1 **Alpha purothionin precursor [Pur-B1] [Triticum aestivum]** 137  
Q9T0P1\_WHEAT (Wheat) AA  
align

Score = 268 bits (686), Expect = 1e-71  
Identities = 124/136 (91%), Positives = 132/136 (96%)

Query: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

MGSKG KGV+VCLLILGLVLEQ+QVEGKSCCR+TLGRNCYNLCR+RGAQKLC+ VCRCK+  
Sbjct: 1 MGSKGFKGVIVCLLILGLVLEQLQVEGKSCCRSTLGRNCYNLCRARGAQKLCAGVCRCKI 60

Query: 61 TSGLSCPKEFKLVLESNSDEPDTIEYCNLGCRSSVCDYIMVNAADDEEMKLYVENCDA 120  
+SGLSCPKEFKLVLESNSDEPDTIEYCNLGCRSSVCDYIMVNAADDEEMKLYVENC DA

Sbjct: 61 SSGLSCKPKGFKLVLESNSDEPDTIEYCNLGCRSSVCDYIMVNAADDEEMKLYVENCADA 120

Query: 121 CVNFCNGDAGLTSlda 136  
CV+FCNGDAGL SLda

Sbjct: 121 CVSFCNGDAGLPSlda 136

tr Q9T0P2 **Beta purothionin precursor [Pur-A1] [Triticum aestivum]** 136  
Q9T0P2\_WHEAT (Wheat) AA  
align

Score = 267 bits (682), Expect = 3e-71  
Identities = 124/136 (91%), Positives = 130/136 (95%)

Query: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60

MGSKGLKGMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+ VCRCKL  
Sbjct: 1 MGSKGLKGMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRARGAQKLCANVCRCKL 60

Query: 61 TSGLSCPKEFKLVLESNSDEPDTIEYCNLGCRSSVCDYIMVNAADDEEMKLYVENCDA 120  
TSGLSCPKEFKLVLESNSDEPDTIEYCNLCRSS+CDYIMVNAADDEEMKLYVE CGDA

Sbjct: 61 TSGLSCPKEFKLVLESNSDEPDTMEYCNLECRSSLCDYIMVNAADDEEMKLYVEQCGDA 120

Query: 121 CVNFCNGDAGLTSlda 136  
CVNFCN DAGLTSlda

Sbjct: 121 CVNFCNADAGLTSlda 136

sp P21742 **Beta-hordothionin precursor [THI1.2] [Hordeum vulgare]** 136  
THNB\_HORVU (Barley) AA  
align

Score = 257 bits (657), Expect = 2e-68

Identities = 119/136 (87%), Positives = 125/136 (91%)

```
Query: 1  MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
          MGSKGLKGMVCLLILGLVLE VQVEGKSCCR+TLGRNCYNLCR RGAQKLC+ CRCKL
Sbjct: 1  MGSKGLKGMVCLLILGLVLEHVQVEGKSCCRSTLGRNCYNLCRVRGAQKLCANACRCKL 60

Query: 61 TSGLSCPFGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120
          TSGL CP FPKLAL SNSDEPDTI+YCNLGCR+S+CDYMNAAADDEEMKLYVE+C DA
Sbjct: 61 TSGLKCPSSFPKLALVSNSDEPDTIDYCNLGCRASMCYMNAAADDEEMKLYVEHCSDA 120

Query: 121 CVNFCNGDAGLTSlda 136
          CVNFCNGD GLTSL A
Sbjct: 121 CVNFCNGDVGLTSLTA 136
```

tr Q9ZNY5 **Purothionin precursor [Pur-RL] [Secale cereale (Rye)]** 136 AA  
 Q9ZNY5\_SECCE

align

Score = 257 bits (656), Expect = 3e-68  
 Identities = 119/136 (87%), Positives = 127/136 (92%)

```
Query: 1  MGSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKL 60
          MGSKGLKGMVCLLILGLVLEQVQVEGKSCC++TLGRNCYNLCR+RGAQKLC+ CRCKL
Sbjct: 1  MGSKGLKGMVCLLILGLVLEQVQVEGKSCCKSTLGRNCYNLCRTRGAQKLCANFCRCKL 60

Query: 61 TSGLSCPFGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120
          S SCPK FPKLALESNSDEPDT+EYCNLGCRSSVCDYMV+AAAD+EEMKLYVE CGDA
Sbjct: 61 ISSTSCPKEFPKLALESNSDEPDTVEYCNLGCRSSVCDYMVSAADDEEMKLYVERCGDA 120

Query: 121 CVNFCNGDAGLTSlda 136
          CV+FCNGDAGL SL A
Sbjct: 121 CVSFCNGDAGLPSLSA 136
```

sp P01544 **Alpha-1-purothionin precursor (Purothionin A-II)** 126  
 THN1\_WHEAT **(Fragment)** AA  
 [THI1.1] **[Triticum aestivum (Wheat)]** align

Score = 251 bits (640), Expect = 2e-66  
 Identities = 115/125 (92%), Positives = 122/125 (97%)

```
Query: 12 CLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGF 71
          CLLILGLVLEQ+QVEGKSCCR+TLGRNCYNLCR+RGAQKLC+ VCRCK++SGLSCPKGF
Sbjct: 1  CLLILGLVLEQLQVEGKSCCRSTLGRNCYNLCRARGAQKLCAGVCRCKISSGLSCPKGF 60

Query: 72 KLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFCNGDAGL 131
          KLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENC DACV+FCNGDAGL
Sbjct: 61 KLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCADACVSFCNGDAGL 120

Query: 132 TSLDA 136
          SLDA
Sbjct: 121 PSLDA 125
```

tr Q9S6Y2 **Alpha purothionin (Fragment) [Pur-B1] [Triticum aestivum** 90 AA  
 Q9S6Y2\_WHEAT (Wheat)] align

Score = 181 bits (460), Expect = 2e-45  
 Identities = 83/89 (93%), Positives = 86/89 (96%)

Query: 48 AQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD 107  
 AQKLC+ VCRCK+ SGLSCP KGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD  
 Sbjct: 1 AQKLCAGVCRCKIASGLSCP KGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD 60

Query: 108 EEMKLYVENCGDACVNFCNGDAGLTSLDA 136  
 EEMKLYVENCGDACV+FCNGDAGL SLDA  
 Sbjct: 61 EEMKLYVENCGDACVSFCNGDAGLPSLDA 89

tr Q9S9D7 **Thionin [Hordeum vulgare (Barley)]** 137 AA  
 Q9S9D7\_HORVU align

Score = 155 bits (391), Expect = 2e-37  
 Identities = 73/135 (54%), Positives = 94/135 (69%), Gaps = 2/135 (1%)

Query: 3 SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
 SK +K V++C+LILGLVLEQVQVEGKSCC+ TL RNCYN CR + G++ +C+ CRCK+  
 Sbjct: 4 SKSIKSVVICVLILGLVLEQVQVEGKSCCKDTLARNCYNTCRFAGGSRPVCAGACRCKII 63

Query: 62 SGLSCP KGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
 SG CP +PKL L S EPD +YC +GCR+SVCD M N +EMK + C +AC  
 Sbjct: 64 SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCRNSVCDNMDNVFR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136  
 FCN A + S++A  
 Sbjct: 123 ARFCNDGAVIQSVEA 137

sp Q42838 **Thionin BTH7 precursor [Hordeum vulgare** 137 AA  
 THN7\_HORVU (Barley)] align

Score = 154 bits (389), Expect = 3e-37  
 Identities = 71/135 (52%), Positives = 96/135 (70%), Gaps = 2/135 (1%)

Query: 3 SKGLKGVMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
 +K +K V++C+LILGLVLEQVQVEGKSCC+ T GRNCYN CR + G++ +C+T C CK+  
 Sbjct: 4 NKSIIKSVVICVLILGLVLEQVQVEGKSCCKNTTGRNCYNACRFAGGSRPVCATACGCKII 63

Query: 62 SGLSCP KGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
 SG +CP+ +PKL L S EP+ EYC +GCR+SVCD M N + +EMK + C +AC  
 Sbjct: 64 SGPTCPRDYPKLNLLPESGEPNVTEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136  
 FCN + S++A  
 Sbjct: 123 ARFCNDGEVIQSVEA 137

sp P08772 **Leaf-specific thionin DB4 precursor [THI1.3] [Hordeum** 137  
 THN3\_HORVU **vulgare** AA  
**(Barley)]** align

Score = 153 bits (386), Expect = 6e-37

Identities = 72/135 (53%), Positives = 93/135 (68%), Gaps = 2/135 (1%)

Query: 3 SKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
 SK +K V++C+LILGLVLEQVQVEGKSCC+ TL RNCYN C + G++ +C+ CRCK+  
 Sbjct: 4 SKSIKSVVICVLILGLVLEQVQVEGKSCCKDTLARNYNTCHFAGGSRPVCAGACRCKII 63

Query: 62 SGLSCPFGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
 SG CP +PKL L S EPD +YC +GCR+SVCD M N +EMK + C +AC  
 Sbjct: 64 SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCRNSVCDNMDNVFR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136  
 FCN A + S++A  
 Sbjct: 123 ARFCNDGAVIQSVEA 137

sp Q8H0Q5 **Probable leaf thionin precursor [Hordeum vulgare (Barley)]** 137 AA  
 THNX\_HORVU align

Score = 152 bits (383), Expect = 1e-36

Identities = 70/135 (51%), Positives = 95/135 (69%), Gaps = 2/135 (1%)

Query: 3 SKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
 +K +K V++C+LILGLVLEQVQVEGKSCC+ T GRNCYN C + G++ +C+T C CK+  
 Sbjct: 4 NKSISVVICVLILGLVLEQVQVEGKSCCKNTTGRNCYNACHFAGGSRPVCATACGCKII 63

Query: 62 SGLSCPFGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
 SG +CP+ +PKL L S EP+ EYC +GCR+SVCD M N + +EMK + C +AC  
 Sbjct: 64 SGPTCPRDYPKLNLLPESGEPNATEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTSLDA 136  
 FCN + S++A  
 Sbjct: 123 ARFCNDGDVIQSVEA 137

sp P09617 **Leaf-specific thionin precursor (Clones PKG1348, PKG1940,** 137  
 THN5\_HORVU **PKG2872** AA  
**and DG3) [THI1.5] [Hordeum vulgare (Barley)]** align

Score = 152 bits (383), Expect = 1e-36

Identities = 70/135 (51%), Positives = 95/135 (69%), Gaps = 2/135 (1%)

Query: 3 SKGLKGMVCLLILGLVLEQVQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
 +K +K V++C+LILGLVLEQVQVE KSCC+ T GRNCYN CR + G++ +C+T C CK+  
 Sbjct: 4 NKSISVVICVLILGLVLEQVQVEAKSCCKNTTGRNCYNACRFAGGSRPVCATACGCKII 63

Query: 62 SGLSCPFGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
 SG +CP+ +PKL L S EP+ EYC +GCR+SVCD M N + +EMK + C +AC  
 Sbjct: 64 SGPTCPRDYPKLNLLPESGEPNATEYCTIGCRTSVCDNMDNVSR-GQEMKFDMGLCSNAC 122

Query: 122 VNFCNGDAGLTS LDA 136  
FCN + S++A  
Sbjct: 123 ARFCNDGEVIQSVEA 137

sp P09618 **Leaf-specific thionin BTH6 precursor [Hordeum vulgare** 137  
THN6\_HORVU **(Barley)]** AA  
align

Score = 149 bits (376), Expect = 9e-36  
Identities = 71/135 (52%), Positives = 92/135 (67%), Gaps = 2/135 (1%)

Query: 3 SKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLT 61  
SK +K V++C+LIL LVLEQVQVEGKSCC+ TL RNCYN CR + G++ +C+ CRCK+  
Sbjct: 4 SKSIKSVVICVLILVLVLEQVQVEGKSCCKDTLARNCYNTCRFAGGSRPVCAGACRCKII 63

Query: 62 SGLSCPFGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDAC 121  
SG CP +PKL L S EPD +YC +GC +SVCD M N +EMK + C +AC  
Sbjct: 64 SGPKCPSDYPKLNLLPESGEPDVTQYCTIGCTNSVCDNMDNVFR-GQEMKFDMLCSNAC 122

Query: 122 VNFCNGDAGLTS LDA 136  
FCN A + S++A  
Sbjct: 123 ARFCNDGAVIQSVEA 137

tr Q8LT02 **Leaf thionin Asthi2 [Asthi2] [Avena sativa** 137 AA  
Q8LT02\_AVES **(Oat)]** align

Score = 145 bits (367), Expect = 1e-34  
Identities = 71/128 (55%), Positives = 93/128 (72%), Gaps = 3/128 (2%)

Query: 1 MGS-KGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCR 58  
MGS KGLK V++C+L+LG+VLE VQVEGKSCC+ T RNCYN+CR G + +C+T CRC  
Sbjct: 1 MGSIKGLKSVVICVLVLGIVLEHVQVEGKSCCKDTTARNCYNVCRIPTPRPVCATTCCR 60

Query: 59 KLTSGLSCPFGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCG 118  
K+ SG CPK +PKL + ++ P+ IE+CN+GC SSV D M N A + EE ++ VE CG  
Sbjct: 61 KIISGNKCPKDYKLGDLGAGTPNAIEFCNMGMCMSSVRDNM-NNAYNIEEKEIDVELCG 119

Query: 119 DACVNFCN 126  
A +FCN  
Sbjct: 120 TAYASFCN 127

tr Q8LSZ9 **Thionin Asthi5 [Asthi5] [Avena sativa** 136 AA  
Q8LSZ9\_AVES **(Oat)]** align

Score = 138 bits (348), Expect = 2e-32  
Identities = 69/135 (51%), Positives = 89/135 (65%), Gaps = 3/135 (2%)

Query: 3 SKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLT 61  
+KGLK V++C+L+LGLVL QVQVEGKSCC +T RNCYN+CR G + +C+++C CK+

```

Sbjct: 4      TKGLKCVVLVVLGLVLGQVQVEGKSCCPSTSARNCYNVCRLTGTSRPRCASLCGCKIV 63
Query: 62     SGLSCPKGFPKLALESNSDEPDITIEYCNLGRSSVCDYMVNAAADDEEMKLYVENCGDAC 121
              G +CP G+ KL L   S EPD  EYC +GC +SVCD M N       +EMK+ +   C   C
Sbjct: 64     DG-TCPDGYSKLHLLLESGE PDVTEYCTIGCMTSVCDNMDN-VIHGQEMKIDMLLCNKEC 121
Query: 122    VNFCNGDAGLTSLDA 136
              V FCN  A + S   A
Sbjct: 122    VRFCNKGAVIPSFQA 136

```

|    |               |                                       |        |
|----|---------------|---------------------------------------|--------|
| tr | <u>Q8LT00</u> | Thionin Asthi4 [Asthi4] [Avena sativa | 142 AA |
|    | Q8LT00 AVESA  | (Oat)]                                | align  |

Score = 134 bits (338), Expect = 2e-31  
Identities = 67/139 (48%), Positives = 88/139 (63%), Gaps = 5/139 (3%)

Query: 3 SKGLKGMVCLLILGLVLVEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLT 61  
SKG+K ++C L+LGLVLVEQVQVEGKSCC++T NCYN+CR GA + ++C C CKL  
Subject: 4 SKGIKSAVICFLMLGLVLVEQVQVEGKSCCKSTTAINCYNVCRLAGAPRPPVAGPCGCKLL 63

Query: 62 SGLSCP KGF PKLALESNSDEPDTIEYCNLGCRSSVCDYMVNA----AADDEEMKLYVENC 117  
+CP +PK L S E D EYC +GC +SVCD + NA +EM + ++ C  
Subject: 64 DVTTCPSDWPKQHLLSEYGEADAAEYCTIGCMTSVCDNIGNAMFAPIVRGQEMNIDMQVC 123

```
Query: 118 GDACVNFCNGDAGLTSLDA 136
      +ACV FCN  A   S+ A
Sbjct: 124 NNACVRFCNKGAVNPSVGA 142
```

[illegible]

Score = 134 bits (336), Expect = 4e-31  
Identities = 68/135 (50%), Positives = 87/135 (64%), Gaps = 10/135 (7%)

Query: 2 GSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60  
G KGL+ +VCLL+LGLVLEQVQVEG C C+N C + C+ C C+L  
Sbjct: 4 GKKGLESAIVCLLVGLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVFQCADFCACRL 63

Query: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENC GDA 120  
+GL+ + +SDEP+ IEYC+LGCRSSVCD +++ A + EEMKLYV+ CGDA  
Sbjct: 64 PAGLASLR-----SSDEPNAIEYCSLGCRSSVCDNVISTADNTEEMKLYVKRC GDA 114

Query: 121 CVNFCNGDAGLTSLD 135  
C +FC GD L SLD  
Sbjct: 115 CDSFCKGDTLLASLD 129

```

sp   Q05806      Type V thionin precursor [TTHV] [Triticum aestivum]      131
     THN5_WHEAT (Wheat)]
     .
     align

```



Score = 130 bits (326), Expect = 6e-30

Identities = 69/136 (50%), Positives = 86/136 (62%), Gaps = 11/136 (8%)

```

Query: 2   GSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60
          G KGL+ +VCLL+LGLVLEQVQVEG C C+N C + C+ C C+L
Sbjct: 4   GQKGLESAIVCLLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVTFQCADFCACRL 63

Query: 61  TSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADD-EEMKLYVENCGD 119
          +GL+ + +SDEP+ IEYC+LGRSSVCD M+N A + EEMKLYV+ CG
Sbjct: 64  PAGLASVR-----SSDEPNIEYCSLGRSSVCDNMINTADNSTEEMKLYVKRCGV 114

Query: 120 ACVNFCNGDAGLTS LD 135
          AC +FC GD L SLD
Sbjct: 115 ACDSFCKGDTLLASLD 130

```

tr Q8LT01 **Leaf thionin Asthi3 [Asthi3] [Avena sativa** 137 AA  
Q8LT01\_AVES **(Oat)]** align

Score = 129 bits (325), Expect = 8e-30

Identities = 64/138 (46%), Positives = 89/138 (64%), Gaps = 3/138 (2%)

```

Query: 1   MGS-KGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCR 58
          MGS KGL+ +++C+LI+G+VLEQVQVEG +CC+ + RNCYN+CR G +C+ +CRC
Sbjct: 1   MGSIKGLRSLIMCVLIVGIVLEQVQVEGNTCKDDIARNCYNVCRIPTPTFICANMCRC 60

Query: 59  KLTSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADDEEMKLYVENCG 118
          +T CP +PKL + ++ P+ IE+CN+GC SS+C M N A EE + E C
Sbjct: 61  IITRNECPNDYPKLQSDLDAGTPNAIEFCNMGMSSICGNMKN-AYPGEKENDKEFCS 119

Query: 119 DACVNFCNGDAGLTS LDA 136
          AC FCN TS+ A
Sbjct: 120 IACARFCNKITVSTSVAA 137

```

tr Q38770 **Type V Thionin precursor [AthV1] [Aegilops tauschii** 131  
Q38770\_AEGTA **(Tausch's** AA  
**goatgrass) (Aegilops squarrosa)]** align

Score = 129 bits (325), Expect = 8e-30

Identities = 69/136 (50%), Positives = 86/136 (62%), Gaps = 11/136 (8%)

```

Query: 2   GSKGLKGMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKL 60
          G KGL+ +VCLL+LGLVLEQVQVEG C C+N C + C+ C C+L
Sbjct: 4   GQKGLESAIVCLLVLEQVQVEGVDCGANPFKVACFNSCLLGPSTVTFQCADFCACRL 63

Query: 61  TSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADD-EEMKLYVENCGD 119
          +GL+ + +SDEP+ IEYC+LGRSSVCD M+N A + EEMKLYV+ CG
Sbjct: 64  PAGLASVR-----SSDEPNIEYCSLGRSSVCDNMNIRADNSTEEMKLYVKRCGV 114

Query: 120 ACVNFCNGDAGLTS LD 135
          AC +FC GD L SLD
Sbjct: 115 ACDSFCKGDTLLASLD 130

```

tr Q5Z4W6 Putative thionin Osth1 [OSJNBb0071G09.18] [Oryza sativa 135  
 Q5Z4W6\_ORYSA (japonica AA  
 cultivar-group)] align

Score = 108 bits (270), Expect = 2e-23

Identities = 56/134 (41%), Positives = 79/134 (58%), Gaps = 5/134 (3%)

Query: 4 KGLKGVMLVCLLILGLVL--EQVQVEGKSCCRTLGRNCYNLCRSRGAQK-LCSTVCRCKL 60  
 + +K ++VC+L+LGLVL E +QVE KSCC +T RN YN CR GA + C + CK+  
 Sbjct: 2 EAVKSLIVCVLVLGLVLQHEHIQVEAKSCCPSTTARNIYNSCRFTGASRDKCKISGCKI 61

Query: 61 TSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
 G C F L +S+E D +++C LGC SSVC M N A +EE V+ C +A  
 Sbjct: 62 VDG-KCKPPFIHHTLHPDSEESDVLDFCKLGCTSSVCSNM-NTFAGNEEGNHAVDRCNEA 119

Query: 121 CVNFCNGDAGLTSL 134  
 C FC +A + ++  
 Sbjct: 120 CYRFTNEAEIVTV 133

tr Q5Z5V1 Putative thionin Osth1 [OSJNBa0020P04.23] [Oryza sativa 135  
 Q5Z5V1\_ORYSA (japonica AA  
 cultivar-group)] align

Score = 106 bits (264), Expect = 9e-23

Identities = 54/134 (40%), Positives = 82/134 (60%), Gaps = 5/134 (3%)

Query: 4 KGLKGVMLVCLLILGLVLEQ--VQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
 KG+K +++C+L+LGLVL+Q ++V KSCC TT RN YN CR + G ++ CS + CK+  
 Sbjct: 2 KGVKSLIMCVLVLGLVLQQETIKVGAKSCCPSTTARNIYNACRFAHGTRERCSKLSGCKI 61

Query: 61 TSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
 G C + L ++E D +++C LGC SSVC +N +EE VE C +A  
 Sbjct: 62 VDG-KCKPPYIHHTLHDFAEELDVLDVDFCM LGCTSSVCS-NINTCKLNEEGNGAVERCNEA 119

Query: 121 CVNFCNGDAGLTSL 134  
 C +FCN +A + ++  
 Sbjct: 120 CYHFCNKEADIVTI 133

tr Q5Z434 Putative thionin Osth1 [OSJNBa0061G23.50] [Oryza sativa 135  
 Q5Z434\_ORYSA (japonica AA  
 cultivar-group)] align

Score = 105 bits (261), Expect = 2e-22

Identities = 51/129 (39%), Positives = 80/129 (61%), Gaps = 5/129 (3%)

Query: 4 KGLKGVMLVCLLILGLVLEQ--VQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
 +G+K +++C+L+LGLVL+Q +QVE KSCC +T RN YN CR + G++ C+ + CK+  
 Sbjct: 2 EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAGGSRDTCAKLSGCKI 61

Query: 61 TSGLSCPFGFPKLALESNSDEPDTEIYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
 G +C + L ++E + +++C LGC SSVC M + + +EE V+ C DA  
 Sbjct: 62 VDG-NCKPPYVHHTLHPEAESEVLDVDFCKLGCASSVCSTM-STLSSNEEANYAVDRCNDA 119

Query: 121 CVNFCNGDA 129  
C FC +A  
Sbjct: 120 CHRFACTKEA 128

tr Q5Z4S4 Putative thionin Osth1 [P0597A07.39] [Oryza sativa 135  
Q5Z4S4\_ORYSA (japonica AA  
cultivar-group)] align

Score = 102 bits (255), Expect = 1e-21  
Identities = 51/129 (39%), Positives = 79/129 (60%), Gaps = 5/129 (3%)

Query: 4 KGLKGVMLVLLILGLVLEQ--VQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
+G+K +++C+L+LGLVL+Q +QVE KSCC +T RN YN CR + G++ C+ + CK+  
Sbjct: 2 EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAAGSRNTCAKLSGCKI 61

Query: 61 TSGLSCPFGFPKLALESNSDEPDITIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
G +C + L ++E + +++C LGC SSVC M A +EE V+ C +A  
Sbjct: 62 VDG-NCEPPYVHHTLHPEAESEVVDVCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129  
C FC +A  
Sbjct: 120 CRRFACTKEA 128

tr Q8LT04 Thionin Osth1 [Osth1] [Oryza sativa (japonica 135  
Q8LT04\_ORYSA cultivar-group)] AA  
align

Score = 102 bits (254), Expect = 1e-21  
Identities = 51/129 (39%), Positives = 79/129 (60%), Gaps = 5/129 (3%)

Query: 4 KGLKGVMLVLLILGLVLEQ--VQVEGKSCCRTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
+G+K +++C+L+LGLVL+Q +QVE KSCC +T RN YN CR + G++ C+ + CK+  
Sbjct: 2 EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAAGSRDTCALSGCKI 61

Query: 61 TSGLSCPFGFPKLALESNSDEPDITIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
G +C + L ++E + +++C LGC SSVC M A +EE V+ C +A  
Sbjct: 62 VDG-NCKPPYVHHTLHPEAESEVVDVCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129  
C FC +A  
Sbjct: 120 CRRFACTKEA 128

tr Q5Z4K0 Thionin Osth1 [OSJNBa0022006.36] [Oryza sativa 135  
Q5Z4K0\_ORYSA (japonica AA  
cultivar-group)] align

Score = 101 bits (252), Expect = 2e-21  
Identities = 50/129 (38%), Positives = 79/129 (60%), Gaps = 5/129 (3%)

Query: 4 KGLKGVMLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
 +G+K +++C+L+LGLVL+Q +QVE KSCC +T RN YN CR + G++ C+ + CK+  
 Sbjct: 2 EGVKSLIMCMLVLGLVLQQEKIQVEAKSCCPSTTARNVYNSCRFAAGSRDTCALSGCKI 61

Query: 61 TSGLSCPKGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDA 120  
 G +C + L ++E + +++C LGC SSVC M + +EE V+ C +A  
 Sbjct: 62 VDG-NCKPPYVHHTLHPEAESEVVD FCKLGCASSVCSTM-STLFGNEEANHAVDRCNEA 119

Query: 121 CVNFCNGDA 129  
 C FC +A  
 Sbjct: 120 CRRFCTKEA 128

tr Q9S975 Crambin PRECURSOR=THIONIN variant THI2CA11 (Fragment) 130  
 Q9S975\_CRAAB [Crambe AA  
 abyssinica (Abyssinian crambe)] align

Score = 90.1 bits (222), Expect = 7e-18  
 Identities = 48/120 (40%), Positives = 68/120 (56%), Gaps = 5/120 (4%)

Query: 9 VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSKP 67  
 V++ +LI+ LV+ Q QVE KSCC + RN YN+CR G + +C+++ CK+ S CP  
 Sbjct: 2 VILSVLIMSLVIAQNQVEAKSCCPSITARNTYNVCRLPGTTPRPVCASISGCKILSVTKCP 61

Query: 68 KGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD--EEMKLYVENCGDACVNFC 125  
 P L+++ D + EYC LGC SSVC + D E + V C +AC NFC  
 Sbjct: 62 SNLPYENLNKNSGDVVN--EYCKLGCASSVCGALTTLQNSDASEVVDGAVAQCTNACS NFC 119

tr Q43225 Thionin class 1 precursor [Thi1-2] [Tulipa gesneriana] 114  
 Q43225\_TULGE (Tulip) AA  
 align

Score = 90.1 bits (222), Expect = 7e-18  
 Identities = 51/121 (42%), Positives = 64/121 (52%), Gaps = 22/121 (18%)

Query: 10 MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSKP 68  
 M+ ++ILGLV+ Q QVE KSCCR T RNCYN+CR G + +C+ C CK+ S CP  
 Sbjct: 1 MMVVVILGLVVAQTQVEAKSCCRNTTARNYCNVCRLPGTTPRPVCAATCDCKIISGKCPP 60

Query: 69 GFPKLALESNSDEPDTIEYCNLGRSSVCDY--MVNAAAD--DEEMKLYVENCGDACVNFC 124  
 G+ K LGC SS C +V+ A D E MK VE C +AC  
 Sbjct: 61 GYEK-----LGCASSTCSTIDVVDEALDVAKEVMKEAVERCANNACSEV 103

Query: 125 C 125  
 C  
 Sbjct: 104 C 104

sp Q9SBK8 Thionin precursor [THI2] [Brassica rapa subsp. pekinensis] 133  
 THN\_BRARP (Chinese AA  
 cabbage) (Celery cabbage)] align

Score = 89.4 bits (220), Expect = 1e-17

Identities = 48/121 (39%), Positives = 67/121 (54%), Gaps = 4/121 (3%)

```

Query: 7  KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKL-CSTVCRCKLTSGLS 65
          K V++ ++I+ LV+ Q QVE K CC  T+ RN YN CR  GA    C+ +  CK+ SG +
Sbjct: 4  KTVILGVIIMSLVMAQNQVEAKICCPRTIDRNIYNACRLTGASMTNCANLSGCKIVSGTT 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDE-EMKLYVENCGDACVNF 124
          CP G+  L++ D +  EYC LGC SSVC +      +  + V C +AC NF
Sbjct: 64  CPPGYTHDILQNYGDAVN--EYCKLGCASSVCGALTTLKNSMQVNCEGAVSQCTNACSNF 121

Query: 125 C 125
          C
Sbjct: 122 C 122

```

```

tr Q43224      Thionin class 1 precursor [Thi1-1] [Tulipa gesneriana] 121
Q43224_TULGE (Tulip) ] AA
align

```

Score = 87.8 bits (216), Expect = 3e-17

Identities = 51/118 (43%), Positives = 69/118 (58%), Gaps = 9/118 (7%)

```

Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGA-QKLCSTVCRC-KLTSGLS 67
          M+ ++ILGLV+ Q QVE KSCCRTT  RNCYN+CR  G  Q LC+  C C  +T+G +CP
Sbjct: 1  MMVVVILGLVVAQTQVEAKSCCRTTAARNCYNVCRLGGTPQTL CARTCDCIHITG-NCP 59

Query: 68  KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFC 125
          + PKL  S++  ++  L      V D +++ A  E MK  VE C +AC  C
Sbjct: 60  RSHPKLGSASSTSTTNVDDEAL----DVVDEVLDVA--KEAMKEAVERCINNACSEVC 111

```

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tr Q9S976      Crambin PRECURSOR=THIONIN variant THI2CA10 (Fragment) 134
Q9S976_CRAAB [Crambe
abyssinica (Abyssinian crambe)] AA
align

```

Score = 86.3 bits (212), Expect = 1e-16

Identities = 47/122 (38%), Positives = 68/122 (55%), Gaps = 5/122 (4%)

```

Query: 7  KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLS 65
          K V++ +LI+ LV+ Q QVE KSCC +  RN YN+CR  G  + +C+T+  C + S  +
Sbjct: 4  KSVILSVLIISLVMAQNQVEAKSCCPSITARNTYNICRLPGTPRPVCATLSGCIIQSDST 63

Query: 66  CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKL--YVENCGDACVN 123
          C  +P  L+++ D +  EYC LGC SSVC +      D  +  V C +AC N
Sbjct: 64  CKPPYPYGDLKNSGDAVN--EYCKLGCASSVCGSLATFQNSDASKIVDGAQAQCTNACSN 121

Query: 124 FC 125
          FC
Sbjct: 122 FC 123

```

```

tr Q43226      Thionin class 1 precursor [Thi1-3] [Tulipa gesneriana] 107

```

Q43226\_TULGE (Tulip)]

AA  
align

Score = 85.9 bits (211), Expect = 1e-16  
Identities = 47/117 (40%), Positives = 61/117 (51%), Gaps = 21/117 (17%)

Query: 10 MVCLLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCP 68  
M+ ++ILGLV+ Q QVE KSCC T RNCYN+CR G + +C+ C CK+ S CP  
Sbjct: 1 MMVVVILGLVVAQTQVEAKSCCRNTTARNCYNVCRLPGTPRPVCAATCDCKIISGKCPP 60

Query: 69 GFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADDEEMKLYVENCGDACVNFC 125  
G+ KL S V D ++ A +E MK VE C +AC C  
Sbjct: 61 GYEKLGF-----SDVADEALDVA--EEVMKEAVERCINNACSEVC 97

tr Q9S981 Crambin PRECURSOR=THIONIN variant THI2CA2 (Fragment) 134  
Q9S981\_CRAAB [Crambe AA  
abyssinica (Abyssinian crambe)] align

Score = 85.1 bits (209), Expect = 2e-16  
Identities = 48/122 (39%), Positives = 65/122 (52%), Gaps = 5/122 (4%)

Query: 7 KGVMVCLLLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQ-KLCSTVCRCKLTSGLS 65  
K V++ +LI+ LV+ QVE KSCC T + R Y +CR G+ C C SG  
Sbjct: 4 KIVILSVLIMNLVMAHNQVEAKSCCPTPIARKTYVVCRLTGSTIASCIKYSGCITISGTQ 63

Query: 66 CPKGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD--EEMKLYVENCGDACVN 123  
CP G+P L ++ D + EYC LGC SSVC + D E + + V +C +AC N  
Sbjct: 64 CPNGYPHDILGNSGDAVN--EYCKLGCASSVCGALTTLKNSDASEIVNVAHAHCTNACSN 121

Query: 124 FC 125  
FC  
Sbjct: 122 FC 123

tr Q9S974 Crambin PRECURSOR=THIONIN variant THI2CA12 (Fragment) 135  
Q9S974\_CRAAB [Crambe AA  
abyssinica (Abyssinian crambe)] align

Score = 84.3 bits (207), Expect = 4e-16  
Identities = 48/123 (39%), Positives = 68/123 (55%), Gaps = 6/123 (4%)

Query: 7 KGVMVCLLLILGLVLEQVQVEG-KSCCRTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGL 64  
K V++ +LI+ LV+ Q QVE KSCC T RN +++CR G LC+ + CK+ S  
Sbjct: 4 KTVILSVLIMSLVMAQNQVEAAKSCCPTKSARNTFDVCRLTGTSMLCAAISECKILSVT 63

Query: 65 SCPKGFPKLALESNSDEPDTIEYCNLGRSSVCDYMNAAADD--EEMKLYVENCGDACV 122  
CP P L+++ D + EYC LGC SSVC + + D E + V C +AC  
Sbjct: 64 KCPSNLPYDNLKNSGDAVN--EYCKLGCASSVCGSLTTLQSSDASEIVDGAVAQCTNACS 121

Query: 123 NFC 125  
+FC  
Sbjct: 122 DFC 124

tr Q9S9A0 **Thionin [Viscum album (European mistletoe)]** 114 AA  
Q9S9A0\_VISAL  align

Score = 83.2 bits (204), Expect = 8e-16  
 Identities = 44/118 (37%), Positives = 64/118 (53%), Gaps = 23/118 (19%)

Query: 9 VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLS 67  
 +++ +L+LG +L VE KSCCR T GRNCYN CR G + +C+++C CK+ SG CP  
 Sbjct: 9 MLLLVLVLLGALLVS-SVESKSCCRNTTGRNCYNACRVPGTTPRVCASLDCCKIISGSKCP 67

Query: 68 KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFC 125  
 +P+ YC LGC+S+ C N+ D E ++ C AC + C  
 Sbjct: 68 ADYPRF-----YCTLGCQSTQC---ANSNGDAEAVR-----CKTACSDL 104

sp Q42597 **Thionin 2.2 precursor [THI2.2] [Arabidopsis thaliana** 134  
THN22\_ARATH **(Mouse-ear** AA  
**cross)]** align

Score = 82.8 bits (203), Expect = 1e-15  
 Identities = 51/124 (41%), Positives = 64/124 (51%), Gaps = 5/124 (4%)

Query: 7 KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLS 65  
 K V+ LLI+ LVL Q+QVE K CC T R+ Y +C S +Q C +CK TS  
 Sbjct: 4 KTVISSLLIMSLVLAQIQVEAKICCPKDDRSVYFVCMLSVSSQFYCLLKSCKNTSQT 63

Query: 66 CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKL--YVENCGDACVN 123  
 CP G+ LE++ D + EYC LGC SSV C + D L VE C AC +  
 Sbjct: 64 CPPGYTNDILENSGDAVN--EYCKLGCASSVCGALTTLQNFDTSKVLSEAVEQCTKACSS 121

Query: 124 FCNG 127  
 C G  
 Sbjct: 122 VCTG 125

tr Q43227 **Thionin class 1 precursor (Fragment) [Thi1-4] [Tulipa** 112  
Q43227\_TULGE **gesneriana** AA  
**(Tulip)]** align

Score = 82.0 bits (201), Expect = 2e-15  
 Identities = 46/113 (40%), Positives = 60/113 (52%), Gaps = 13/113 (11%)

Query: 16 LGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCP 74  
 LGLV+ Q QV+ KSCC +T RNCYN+CR G + +C+ C CK+ +G CP +PKL  
 Sbjct: 1 LGLVVAQTQVDAKSCCPSTAARNCYNVCRFPGTTPRVCAATCGCKIITGTCKPPDYPKLG 60

Query: 75 LES--NSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFC 125  
 + NSD D V D ++ A E MK VE C +AC C  
 Sbjct: 61 WSTFQNSDVADK-----ALDVVDEALHVA--KEVMKEAVERCINNACSEVC 103

tr Q9S980 **Crambin PRECURSOR=THIONIN variant THI2CA3 (Fragment)** 133  
Q9S980\_CRAAB **[Crambe** AA

**abyssinica (Abyssinian crambe)]**align

Score = 80.9 bits (198), Expect = 4e-15

Identities = 49/122 (40%), Positives = 67/122 (54%), Gaps = 6/122 (4%)

Query: 7 KGVMLCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLS 65  
 K V++ +LI+ LV+ Q QVE KSCC T R YN CR+ G + +C+ + CK+

Sbjct: 4 KTVILSVLIMTLVMAQNQVEAKSCCPTMAARIQYNACRALGTPRPVCAALSGCKILDVTK 63

Query: 66 CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADD--EEMKLYVENCGDACVN 123  
 CP + L+S SD + EYC LGC SSVC + N D E + V C +AC +

Sbjct: 64 CPPDYRYDILKS-SDALN--EYCKLGCASSVCGALANLQNSDASEIVNGAVVQCNNACSS 120

Query: 124 FC 125  
 FC

Sbjct: 121 FC 122

tr Q9S977 **Crambin PRECURSOR=THIONIN variant THI2CA9 (Fragment)** 135  
 Q9S977\_CRAAB **[Crambe** AA  
**abyssinica (Abyssinian crambe)]** align

Score = 80.5 bits (197), Expect = 5e-15

Identities = 47/123 (38%), Positives = 67/123 (54%), Gaps = 6/123 (4%)

Query: 7 KGVMLCLLILGLVLEQVQVEG-KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGL 64  
 K V++ +LI+ LV+ Q QVE KSC T RN +++CR G LC+ + CK+ S

Sbjct: 4 KTVILSVLIMSLVMAQNQVEAAKSCYPTKSARNTFDVCRLTGTSMLGLCAAISECKILSVT 63

Query: 65 SCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADD--EEMKLYVENCGDACV 122  
 CP P L+++ D + EYC LGC SSVC + + D E + V C +AC

Sbjct: 64 KCPSNLPYNNLKNSGDAVN--EYCKLGCASSVCGSLTTLQSSDASEIVDGAVAQCTNACS 121

Query: 123 NFC 125  
 +FC

Sbjct: 122 DFC 124

sp Q42596 **Thionin 2.1 precursor [THI2.1] [Arabidopsis thaliana** 134  
 THN21\_ARATH **(Mouse-ear** AA  
**cross)]** align

Score = 79.3 bits (194), Expect = 1e-14

Identities = 45/119 (37%), Positives = 66/119 (54%), Gaps = 6/119 (5%)

Query: 9 VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPK 68  
 +++ LLI+ LV+ QVQVE K CC + RN Y++CR R ++ C V C+ + +CP+

Sbjct: 6 LILSLLIMSLVMAQVQVEAKICCPSNQARNGYSVCRIRFSKGRCMQVSGCQNSD--TCPR 63

Query: 69 GFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADD--EEMKLYVENCGDACVNFC 125  
 G+ LE+++D T E+C LGC +SVC M D E + E C C FC

Sbjct: 64 GWVNAILENSADA--TNEHCKLGCETSVCGAMNTLQNSDASEIVNGASEQCAKGCSIFC 120



sp P01538 **Viscotoxin A3 precursor [THI2.1] [Viscum album (European 111 AA**  
**THN3\_VISAL mistletoe)]**

align

Score = 77.4 bits (189), Expect = 4e-14

Identities = 45/118 (38%), Positives = 61/118 (51%), Gaps = 22/118 (18%)

Query: 9 VMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSGP 67  
 +++ +L+LG +L QVE KSCC T GRN YN CR GA + C+ + CK+ SG +CP  
 Sbjct: 9 LVLLVLLLLGALLVS-QVESKSCCPNTTGRNIYNACRLTGAPRPTCAKLSGCKIISGSTCP 67

Query: 68 KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNF 125  
 +PK YC LGC SS C N+ D E ++ C AC + C  
 Sbjct: 68 SDYPKF-----YCTLGCESSQC--ATNSNGDAEAVR-----CKTACSDL 105

tr Q9S9A2 **Viscotoxin A [Viscum album (European 111 AA**  
Q9S9A2\_VISAL **mistletoe)]**

align

Score = 77.4 bits (189), Expect = 4e-14

Identities = 47/126 (37%), Positives = 60/126 (47%), Gaps = 24/126 (19%)

Query: 4 KGLKGVMLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQ-KLCSTVCRCKLTS 62  
 +G + + LL+ LV+ V E KSCC T GRN YN CR GA C+ + CK+ S  
 Sbjct: 5 RGSSFLFLVLLLGALVVSNNV--ESKSCCPNTTGRNIYNACRLTGAPCPTCAKLSGCKIIS 62

Query: 63 GLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACV 122  
 G +CP +PK YC LGC SS C N D E ++ C AC  
 Sbjct: 63 GSTCPSDYPKF-----YCTLGCESSQC---ANTNGDAEAVR-----CKTACS 101

Query: 123 NFCNGD 128  
 + CN D  
 Sbjct: 102 DLCNND 107

tr Q9S9A1 **Thionin [Viscum album (European mistletoe)] 115 AA**  
Q9S9A1\_VISAL

align

Score = 73.6 bits (179), Expect = 6e-13

Identities = 43/130 (33%), Positives = 63/130 (48%), Gaps = 23/130 (17%)

Query: 4 KGLKGVMLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSG 63  
 +G + + LL+L L VE K CCR G+ CYNLC + + + C+ C CK SG  
 Sbjct: 2 EGARASSLLLLLLLLLGALVVYDVESKICCRAPAGKKCYNLCTALLSSETCANTCYCKDVSG 61

Query: 64 LSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVN 123  
 +CP +P YCNLGC+SS+C A ++ E + C AC +  
 Sbjct: 62 ETCPADYPAF-----YCNLGCQSSLG-----AKSNGEAEAV---RCMTACSD 100

Query: 124 FCNGDAGLTS 133  
 C +G+T+  
 Sbjct: 101 LCG--SGITA 108

tr Q9S979 **Crambin PRECURSOR=THIONIN variant THI2CA5 (Fragment)** 118  
 Q9S979\_CRAAB [**Crambe** AA  
**abyssinica (Abyssinian crambe)**] align

Score = 73.2 bits (178), Expect = 8e-13  
 Identities = 41/112 (36%), Positives = 58/112 (51%), Gaps = 4/112 (3%)

Query: 22 QVQVEGKSCCRTTLGRNCYNLCRSRG-AQKLCSTVCRCKLTSGLSCKPGFPKLALESNSD 80  
 Q QVE CC T R+ +N+CR G A+ +C+T C + G +CP + L++++  
 Sbjct: 1 QNQVEANICCPNTTARSNFVNCRLPGTAEPICATDTGCIIIPGATCPGDYANNILKNSAQ 60

Query: 81 EPDTIEYCNLGCRSSVCDYMNAAADD--EEMKLYVENCGDACVNFC-NGDA 129  
 EYC GC SSVC + N D E + V C +AC +FC NG A  
 Sbjct: 61 GNAVNEYCKWGCASSVCGALTNLQNSDAREIVNGAVRQCTNACFDCTNGSA 112

sp P08943 **Viscotoxin B precursor, (Fragment) [THI2.2] [Viscum album** 103  
 THNB\_VISAL (**European** AA  
**mistletoe)**] align

Score = 72.4 bits (176), Expect = 1e-12  
 Identities = 42/113 (37%), Positives = 58/113 (51%), Gaps = 24/113 (21%)

Query: 25 VEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCKPGFPKLALESNSDEPD 83  
 VE KSCC T GRN YN CR G+++ C+++ CK+ S +CP +PK  
 Sbjct: 4 VESKSCCPNTTGRNIYNTCRLGGGSRERCASLSGCKIISASTCPSDYPKF----- 53

Query: 84 TIEYCNLGCRSSVCDYMV---NAAADDEEMKLYVENCGDACVNFCNGDAGLTS 133  
 YC LGC+SS C + N+ D E ++ C AC N C D G+T+  
 Sbjct: 54 ---YCTLGCQSSKCASITTPPNSEVDAEAVR-----CKAACSNLC--DFGVTT 96

sp Q8VZK8 **Probable thionin 2.3 precursor [At2g15010] [Arabidopsis** 135  
 THN23\_ARATH **thaliana** AA  
**(Mouse-ear cress)]** align

Score = 71.6 bits (174), Expect = 2e-12  
 Identities = 45/123 (36%), Positives = 65/123 (52%), Gaps = 6/123 (4%)

Query: 7 KGVMVCLLILGLVLEQVQVEG-KSCCRTTLGRNCYNLCRSRG-AQKLCSTVCRCKLTSGL 64  
 K V+ +L++GLV+ Q+QVE K+CC + R + C S G Q LCS C+ T  
 Sbjct: 4 KTVIFSVLVLMGLVISQIQVEAQKTCPSQSTRKEFEDCISEGNLQILCSAESGCRDITYVG 63

Query: 65 SCPKGFPKLALESNSDEPDIEYCNLGCRSSVCDYMNAAADDE--EMKLYVENCGDACV 122  
 CP GFP +L ++ D + YC LGC SS+C + + D ++ + VE C AC  
 Sbjct: 64 YCPSPGFPYGLTNSGDVNV--YCKLGCVSSLCGALTSLQKLDTSQKVNVAVERCTKACS 121

Query: 123 NFC 125  
 C  
 Sbjct: 122 TIC 124

tr Q41609 **Thionin class 4 precursor [Thi4-1] [Tulipa gesneriana** 124

Q41609\_TULGE (Tulip)]

AA  
align

Score = 71.6 bits (174), Expect = 2e-12

Identities = 44/118 (37%), Positives = 60/118 (50%), Gaps = 6/118 (5%)

Query: 10 MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSGA--QKLCSTVCRCKLTSGLS 67  
 M+ ++ILGLV+ Q QVE KSC +T + CYN CR G + +C+ C CK+ S +CP  
 Sbjct: 1 MMVVVILGLVVAQTQVEAKSCFPSTAACYCYNACRLPGCRPETICAARCGCKIISGNCP 60

Query: 68 KGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFC 125  
 G+ L S S T + V D ++ A E MK VE C +AC C  
 Sbjct: 61 PGYDYENLGSASSTSSTSNVDDEAL--DVVDEALDVA--KEAMKEAVERCINNACSEVC 114

tr Q5Z554 Putative thionin Osth1 [OSJNBa0085C03.20] [Oryza sativa 112  
 Q5Z554\_ORYSA (japonica AA  
 cultivar-group)] align

Score = 71.2 bits (173), Expect = 3e-12

Identities = 46/129 (35%), Positives = 66/129 (50%), Gaps = 28/129 (21%)

Query: 4 KGLKGMVCLLILGLVLEQ--VQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
 +G+K +++C L+LGLVL+Q + VE KSCC +T RN YN CR + G+++ C+ + CK  
 Sbjct: 2 EGVKSLIMCALVLGLVLQEQEKIHVEAKSCCPSTSVRNVYNSCRFAGGSREACAKLSTCKH 61

Query: 61 TSGLSCPCKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDA 120  
 G SC + L L S DT+ A +E + VE C DA  
 Sbjct: 62 FDG-SCQPPYDHLTLHS-----DTV-----AANEGVNAAVERCEDA 96

Query: 121 CVNFCNGDA 129  
 C FC +A  
 Sbjct: 97 CDRFCTNEA 105

sp Q9C8D6 Probable thionin 2.4 precursor [At1g66100] [Arabidopsis 134  
 THN24\_ARATH thaliana AA  
 (Mouse-ear cress)] align

Score = 65.5 bits (158), Expect = 2e-10

Identities = 42/122 (34%), Positives = 57/122 (46%), Gaps = 5/122 (4%)

Query: 7 KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLS 65  
 K ++V +LI+ L + Q QV+ CC + R YN C + G+ C C S +  
 Sbjct: 4 KTLIVSVLIMSLFMAQNQVDANICCPISIQARTFYNACLFAVGSPSSCIRNSSCLDISEST 63

Query: 66 CPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADD--EEMKLYVENCGDACVN 123  
 CP+G+ LE+ D EYC LGC SSVC + D E + VE C AC  
 Sbjct: 64 CPRGYTNDILENTGDA--VTEYCKLGCVSSVCGALTILQNSDASEIVNGEVEKCTMACST 121

Query: 124 FC 125  
 C  
 Sbjct: 122 VC 123

tr Q5Z551 Putative thionin Osth1 [OSJNBa0085C03.23] [Oryza sativa 148 AA  
Q5Z551\_ORYSA (japonica cultivar-group)]  
[align](#)

Score = 61.2 bits (147), Expect = 3e-09  
Identities = 32/75 (42%), Positives = 48/75 (63%), Gaps = 4/75 (5%)

Query: 4 KGLKGMVCLLILGLVLEQ--VQVEGKSCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKL 60  
+G+K +++C L+LGLVL+Q + VE KSCC +T RN YN CR + G+++ C+ + CK  
Sbjct: 2 EGVKSLIMCALVLLGLVLQQEKIHVAKSCCPSTSARNVYNCRFAGGSREACAKLSTCKH 61

Query: 61 TSGLSCPFGFPKLAL 75  
G SC + L L  
Sbjct: 62 FDG-SCQPPYDHLTL 75

sp P07504 Thionin [THI1] [Pyrularia pubera (Rabbitwood) (Buffalo nut)] 47 AA  
THN\_PYRPU  
[align](#)

Score = 60.8 bits (146), Expect = 4e-09  
Identities = 24/47 (51%), Positives = 33/47 (70%), Gaps = 2/47 (4%)

Query: 28 KSCCRTTLGRNCYNLCRSRG--AQKLCSTVCRCKLTSGLSCPKGFPG 72  
KSCCR T RNCYN+CR G ++++C+ C CK+ SG +CP +PK  
Sbjct: 1 KSCCRNTWARNCYNVCRLPGTISRCAKCKDCCKIISGTTCPSPDYPK 47

sp P60057 Hellethionin D [Helleborus purpurascens (Purple hellebore)] 46 AA  
THND\_HELPU  
[align](#)

Score = 52.4 bits (124), Expect = 2e-06  
Identities = 22/45 (48%), Positives = 27/45 (59%), Gaps = 1/45 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGFPG 71  
KSCCR TL RNCYN CR + G+Q C +C C + +CP P  
Sbjct: 1 KSCCRNTLARNCYNACRFTGGSQPTCGILCDCIHVTTTTCPSSHP 45

sp P83554 Viscotoxin C1 [Viscum album (European mistletoe)] 46 AA  
THNC\_VISAL  
[align](#)

Score = 50.8 bits (120), Expect = 4e-06  
Identities = 21/46 (45%), Positives = 30/46 (64%), Gaps = 1/46 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCPKGFPG 72  
KSCC T GRN YN CR + G+++ C+ + CK+ S +CP +PK  
Sbjct: 1 KSCCPNTTGRNIYNTCRFAGGSRRERCAKLSGCKIISASTCPSPDYPK 46

sp P01541      **Denclatoxin B [Dendrophthora clavata (Columbian mistletoe)]** 46 AA  
    THN\_DENCL  
align

Score = 50.4 bits (119), Expect = 6e-06  
Identities = 21/44 (47%), Positives = 28/44 (62%), Gaps = 1/44 (2%)

Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCKPKGF 70  
          KSCC TT RN YN+CR G + +C+ + CK+ SG CP G+  
Sbjct: 1 KSCCPTTAARNQYNICRLPGTPRPVCAALSGCKIISGTGCPPGY 44

sp P59358      **Ligatoxin B [Phoradendron liga (Argentine mistletoe)]** 46 AA  
    THNB\_PHOLI  
align

Score = 47.8 bits (112), Expect = 4e-05  
Identities = 20/44 (45%), Positives = 29/44 (65%), Gaps = 1/44 (2%)

Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCKPKGF 70  
          KSCC +T RN YN CR GA + +C+++ CK+ SG +C G+  
Sbjct: 1 KSCCPSTTARNIYNTCRLTGASRSVCASLSGCKIISGSTCDSGW 44

sp P01539      **Phoratoxin [Phoradendron tomentosum (California mistletoe)]** 46 AA  
    THN\_PHOTO  
align

Score = 47.0 bits (110), Expect = 6e-05  
Identities = 20/44 (45%), Positives = 27/44 (60%), Gaps = 1/44 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCKPKGF 70  
          KSCC TT RN YN CR G++ +C+ + CK+ SG C G+  
Sbjct: 1 KSCCPSTTARNIYNTCRFGGSRPVCALSGCKIISGTCDSGW 44

sp P32880      **Viscotoxin A2 [THI2.3] [Viscum album (European mistletoe)]** 46 AA  
    THN2\_VISAL  
align

Score = 47.0 bits (110), Expect = 6e-05  
Identities = 19/41 (46%), Positives = 28/41 (67%), Gaps = 1/41 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCKP 67  
          KSCC T GRN YN CR G++++C+++ CK+ S +CP  
Sbjct: 1 KSCCPNTTGRNIYNTCRFGGGSREVCASLSGCKIISASTCP 41

tr Q5Z553      **Hypothetical protein OSJNBa0085C03.21 [OSJNBa0085C03.21]** 86 AA  
    Q5Z553\_ORYSA    **[Oryza**  
                      **sativa (japonica cultivar-group)]**  
align

Score = 46.6 bits (109), Expect = 8e-05  
Identities = 23/67 (34%), Positives = 33/67 (48%), Gaps = 1/67 (1%)

Query: 63 GLSCPKGFPPKLALESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACV 122  
G C G +++ D +++C LGC SSVC +N A +E + VE C AC  
Sbjct: 14 GSGCQFGLNHRFPWVKA EKSDVLD FCKLGCTSSVCS-KINTFAANEGVNAAVERCEHACD 72

Query: 123 NFCNGDA 129  
FC +A  
Sbjct: 73 RFCTNEA 79

sp P01540 **Ligatoxin A [Phoradendron liga (Argentine mistletoe)]** 46 AA  
THNA\_PHOLI  
align

Score = 45.1 bits (105), Expect = 2e-04  
Identities = 19/44 (43%), Positives = 27/44 (61%), Gaps = 1/44 (2%)

Query: 28 KSCCRTTLGRNCYNLCRSRGAQK-LCSTVCRCKLTSGLSCKPKGF 70  
KSCC +T RN YN CR G + C+++ CK+ SG +C G+  
Sbjct: 1 KSCCPSTTARNIYNTCRLTGTSRPTCASLSGCKIISGSTCBSGW 44

tr Q5Z4S0 **Thionin Osthil-like [P0597A07.48] [Oryza sativa (japonica 84 AA**  
Q5Z4S0\_ORYSA **cultivar-group)]**  
align

Score = 45.1 bits (105), Expect = 2e-04  
Identities = 20/50 (40%), Positives = 29/50 (58%), Gaps = 1/50 (2%)

Query: 80 DEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFCNGDA 129  
+E + +++C LGC SSVC M A +EE V+ C +AC FC +A  
Sbjct: 29 EESEVVD FCKLGCASSVCSTMSTLFA-NEEANHAVDRCNEACRRFCTKEA 77

sp P01537 **Viscotoxin 1-PS [THI2.4] [Viscum album (European 46 AA**  
THN1\_VISAL **mistletoe)]**  
align

Score = 44.3 bits (103), Expect = 4e-04  
Identities = 17/41 (41%), Positives = 27/41 (65%), Gaps = 1/41 (2%)

Query: 28 KSCCRTTLGRNCYNLCR-SRGAQKLCSTVCRCKLTSGLSCKP 67  
KSCC T GR+ Y+ CR G++++C+ + CK+ S +CP  
Sbjct: 1 KSCCPBTTGRBIYBTCRFGGSRZVCARISGCKIISASTCP 41

tr Q5Z557 **Hypothetical protein OSJNBa0085C03.15 [OSJNBa0085C03.15] 78 AA**  
Q5Z557\_ORYSA **[Oryza**  
**sativa (japonica cultivar-group)]**  
align

Score = 44.3 bits (103), Expect = 4e-04  
Identities = 20/59 (33%), Positives = 37/59 (61%), Gaps = 1/59 (1%)

Query: 76 ESNSDEPDTIEYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFCNGDAGLTSL 134  
++ ++E D +++C LGC SSVC +N A +EE V++C +A FC+ +A + ++  
Sbjct: 19 DAPTEESDVLDFCKLGCTSSVCS-TINIFAVNEEGNGAVDSCNNARYRFCFSKEAEVVTV 76

tr Q9S978 Crambin PRECURSOR=THIONIN variant THI2CA7 (Fragment) 51 AA  
Q9S978\_CRAAB [Crambe  
abyssinica (Abyssinian crambe)] align

Score = 42.0 bits (97), Expect = 0.002  
Identities = 19/46 (41%), Positives = 27/46 (58%), Gaps = 2/46 (4%)

Query: 52 CSTVCRCKLTSGLSCKPGFKLALESNSDEPDTIEYCNLGCRSSVC 97  
C+ + CK+ S CP P +L+++ D + EYC LGC SSVC  
Sbjct: 6 CAKLSSCKILSVTKCPANLPYESLKNSGDAVN--EYCM LGCASSVC 49

sp O88745 Scrapie-responsive protein 1 precursor (ScRG-1) [Scrg1] 98 AA  
SCRG1\_MOUSE [Mus  
musculus (Mouse)] align

Score = 35.4 bits (80), Expect = 0.19  
Identities = 26/80 (32%), Positives = 35/80 (43%), Gaps = 17/80 (21%)

Query: 6 LKGVMVCLLILGLVLEQVQVEGK--SCCRTTL-GRNCYNLCRSRGAQKL----- 51  
+K V++ +L L L+LE + SC R L RNC+NL R KL  
Sbjct: 2 MKSVVLVILGLTLLLETQAMPSSRLSCYRKLLKDRNCHNLPEGRADLKLIDANVQHHFWD 61

Query: 52 ---CSTVCRCKLTSGLSCKP 68  
C +C C + L CPK  
Sbjct: 62 GKGCCEMICYCNFSELLCCPK 81

sp P01542 Crambin [THI2] [Crambe abyssinica (Abyssinian crambe)] 46 AA  
CRAM\_CRAAB align

Score = 35.0 bits (79), Expect = 0.25  
Identities = 14/40 (35%), Positives = 24/40 (60%), Gaps = 1/40 (2%)

Query: 29 SCCRTTLGRNCYNLCRSRGA-QKLCSTVCRCKLTSGLSCKP 67  
+CC + + R+ +N+CR G + LC+T C + G +CP  
Sbjct: 2 TCCPSIVARSNENVCRLPGTPEALCATYTGCIIPGATCP 41

sp Q9Z0K6 Scrapie-responsive protein 1 precursor (ScRG-1) [Scrg1] 98 AA  
SCRG1\_RAT [Rattus  
norvegicus (Rat)] align

Score = 34.3 bits (77), Expect = 0.43

Identities = 26/77 (33%), Positives = 32/77 (40%), Gaps = 19/77 (24%)

Query: 11 VCLLILGLVL----EQVQVEGKSCCRTTL-GRNCYNLCRSRGAQKL----- 51  
 V L+ILGL L + + SC R L RNC+NL R KL  
 Sbjct: 5 VVLVILGLTLLLGTQAMPSSRLSCYRKLLKDRNCHNLPEGRADLKLIDENVQHFWEGKG 64

Query: 52 CSTVCRCKLTSGLSCPK 68

C +C C + L CPK

Sbjct: 65 CEMICYCNFSELLCCPK 81

tr Q9LN81 **T12C24.19 [Arabidopsis thaliana (Mouse-ear** 212 AA  
Q9LN81\_ARATH **cross)]** align

Score = 33.9 bits (76), Expect = 0.57

Identities = 34/127 (26%), Positives = 50/127 (38%), Gaps = 24/127 (18%)

Query: 13 LLILGLVLEQVQVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCP----- 67  
 L+++ LV+ + +E ++ L CY C L + L CP  
 Sbjct: 9 LVVMMMLVMGNMLIEAEAVMSFKL---CYGGC-----LVACALIAPPIKKLFCPFLCIK 58

Query: 68 --KGFPKLALESNSDEPD-TIEYCNLGRSSVC-----DYMVNAAADDEEMKLYVENCG 118

K P L+ E+N +E D T YC LGC + C D N A K+ + C

Sbjct: 59 DCKRRPMLSFEANLNEIDQTGSYCELGATDRCVSSSSIDDKGNLLAQTAQKIPFKECY 118

Query: 119 DACVNFC 125

AC+ C

Sbjct: 119 PACLVEC 125

tr Q7Q1J5 **ENSANGP00000014375 (Fragment) [ENSANGG00000011886]** 707  
Q7Q1J5\_ANOGA **[Anopheles** AA  
**gambiae str. PEST]** align

Score = 33.5 bits (75), Expect = 0.74

Identities = 26/99 (26%), Positives = 35/99 (35%), Gaps = 14/99 (14%)

Query: 38 NCYNLCRSRGAQKLCSTVC---RCKLTSGLSCPKGFPKLALESNSDEPD-TIEYCNLGC-- 92  
 N N+C + CS C RC C KG+ + +NS YC C  
 Sbjct: 340 NSKNVCEPK-----CSKGCSNGRCVAPDHCECHKGYIATSSSANSKSSICTPYCKNKCVN 394

Query: 93 -----RSSVCDYMVNAAADDEEMKLYVENCGDACVNFCNG 127

R +VC + D + C DA V+ NG

Sbjct: 395 AYCIRPNVCQCLAGHRFADNSTNVCEPICEDALVDCSNG 433

tr Q8W2K6 **Proteinase inhibitor IIa [PIN2a] [Solanum americanum]** 148 AA  
Q8W2K6\_9SOLN align

Score = 33.1 bits (74), Expect = 0.96

Identities = 37/129 (28%), Positives = 52/129 (39%), Gaps = 12/129 (9%)



Query: 10 MVCLLLILG-LVLEQVQVEGKSCCRTTLGRNCYNLC-RSRGA-QKLCSTVCRCKLTSGLSLSC 66  
 + CLL+LG + L V+ K+C R G Y +C RS G+ QK T C C G +  
 Sbjct: 9 LACLLVLGWMFLLAKHVDAAKACTR-ECGHFSYGICPRSEGSPOKPICTNC-CSGYKGCNY 66

Query: 67 PKGFPKLALESNSDEPDTEIYCNLGCRSSVCDYMNAAADDEEMKLYVENCGDACVNFCN 126  
 L E SD P + C C + + + E K+ ++ G C C  
 Sbjct: 67 YSAKGDLIÇEGESD-PRNPKDCTFECDTQI----AYSKCPRSEGMIIKPTG--CTTCCT 119

Query: 127 GDAGLTSLD 135  
 G G D  
 Sbjct: 120 GYQGCYYFD 128

tr Q8MY77 **Bb-cadherin [BbCad] [Branchiostoma belcheri (Amphioxius)]** 796 AA  
 Q8MY77\_BRABE align

Score = 32.3 bits (72), Expect = 1.6  
 Identities = 29/114 (25%), Positives = 46/114 (39%), Gaps = 13/114 (11%)

Query: 24 QVEGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTS-GLSCPFGFPKLALESNSDEP 82  
 Q +G +C RNCYN G + T ++ +CP+GF ++ E + DE  
 Sbjct: 486 QYKGVNC---DTERNCYNFPCQNGGTCIEGTSPTPSISGRNCTCPQGFSGVSCEDDVDEC 542

Query: 83 DTIEYCNLG-CRSSVCDY--MVNAAADDEEMKLYVENCGD-----ACVNFCNG 127  
 C +G CR+ V Y D + ++NC + C +F G  
 Sbjct: 543 SLPNNCVVGTCRNLVGSYECTCKLGYDGYLCRNVIDNCANNPCDPGQCYSFIGG 596

tr Q8BKK7 **Mus musculus 12 days embryo spinal ganglion cDNA, RIKEN** 947  
 Q8BKK7\_MOUSE **full-length** AA  
**enriched library, clone:D130061K05 product:MEGF11** align  
**PROTEIN (KIAA1781) homolog [2410080H04Rik] [Mus musculus**  
**(Mouse)]**

Score = 32.0 bits (71), Expect = 2.1  
 Identities = 24/84 (28%), Positives = 35/84 (41%), Gaps = 12/84 (14%)

Query: 30 CCRTTLGRNCYNLCRSRGAQKLCSTVCRCK-----LTSGLSCPKGFPKLALESNSDEP 82  
 C +G++C C S K C +C+C+ +T +C GF E P  
 Sbjct: 652 CFPGWIGKDCSQGCPSAFFGKDCGHICQCQNGASCDHITGKCTCRTGFSGRHCEQRC-AP 710

Query: 83 DTIEYCNLGCRSSVCDYMNAAAD 106  
 T Y GC+ +C+ M NA D  
 Sbjct: 711 GTFGY---GCQ-QLCECMNNATCD 730

tr Q95V69 **Cell surface immobilization antigen SerH6 [SerH]** 421  
 Q95V69\_TETTH **[Tetrahymena** AA  
**thermophila]** align

Score = 31.6 bits (70), Expect = 2.8

Identities = 12/37 (32%), Positives = 20/37 (53%)

Query: 7 KGVMVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNLC 43  
 K +++CL+I L++ + G C T G+NC C  
 Sbjct: 4 KTIICLIISQLLVSVISAGGAVTCTGTTGQNCSQAC 40

tr Q60XC0 Hypothetical protein CBG18730 (Fragment) [CBG18730] 1168  
 Q60XC0\_CAEBR [Caenorhabditis  
 briggsae] AA  
align

Score = 31.6 bits (70), Expect = 2.8

Identities = 28/99 (28%), Positives = 39/99 (39%), Gaps = 10/99 (10%)

Query: 36 GRNCYNLCRSRGAQKLCSTVCRCKL---TSGLSCPKGFPKLALESNSDEPDTIEYCNLGC 92  
 G C +C + C+ C CKL T+G SC P+ + + CNL C  
 Sbjct: 545 GEKCEQICPNGLWGVDCAHKCSCKLCDPTTG-SCRCEDPERCSDGPCPDGYYGSQC�NLKC 603

Query: 93 RSSV---CDYMNAAADDEEMKLYVENCGDACVNFCNG 127  
 R CD + + LY +NC +C NF G  
 Sbjct: 604 RMDCLNGRCDPIFGYCTCPDG--LYGQNCCKSCPNTFTG 640

sp P04881 Nucleocapsid protein (Nucleoprotein) [N] [Vesicular 422  
 NCAP\_VSVJO stomatitis AA  
 virus (serotype New Jersey / strain Ogden)] align

Score = 31.2 bits (69), Expect = 3.7

Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
 N+ +PD IEY +L C S + + V ++AD E+  
 Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr Q89779 (strain 09/82-HD-B) nucleoprotein ((strain 06/85-NM-E) 422 AA  
 Q89779\_9RHAB nucleoprotein) ((strain 11/83-CA-B) nucleoprotein,  
 complete cds) ((strain 01/84-SN-P1) nucleoprotein) align  
 ((strain 07/83-GA-P) Phosphoprotein and nucleocapsid  
 genes) [Vesicular stomatitis virus]

Score = 31.2 bits (69), Expect = 3.7

Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
 N+ +PD IEY +L C S + + V ++AD E+  
 Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr Q89594 (strain 10/85-HD-B1) nucleoprotein ((strain 12/82-HD-B) 422 AA  
 Q89594\_9RHAB nucleoprotein) [Vesicular stomatitis virus]

[align](#)

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr [Q89520](#) (strain ../49-UT-B1) nucleoprotein ((strain ../52-GA-P) 422 AA  
Q89520\_9RHAB nucleoprotein) [Vesicular stomatitis virus]

[align](#)

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr [Q89040](#) (strain ../60-PN-B) nucleoprotein [Vesicular stomatitis 422  
Q89040\_9RHAB virus] AA

[align](#)

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr [Q89039](#) (strain 01/85-PN-B1) nucleoprotein [Vesicular stomatitis 422  
Q89039\_9RHAB virus] AA

[align](#)

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLLLSFAVGSSADIEQ 346

tr [Q89038](#) (strain 07/83-NC-P) nucleoprotein [Vesicular stomatitis 422  
Q89038\_9RHAB virus] AA

[align](#)

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLSSFAVGSSADIEQ 346

tr Q89037 (strain 10/82-CR-B) nucleoprotein [Vesicular stomatitis 422  
Q89037\_9RHAB virus] AA  
align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLSSFAVGSSADIEQ 346

tr Q89036 (strain 11/84-HD-B1) nucleoprotein [Vesicular stomatitis 422  
Q89036\_9RHAB virus] AA  
align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLSSFAVGSSADIEQ 346

tr Q89035 (strain 10/84-QM-P) nucleoprotein [Vesicular stomatitis 422  
Q89035\_9RHAB virus] AA  
align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCSLSSFAVGSSADIEQ 346

tr Q89034 (strain 07/84-OA-B) nucleoprotein [Vesicular stomatitis 422  
Q89034\_9RHAB virus] AA  
align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+

Sbjct: 315 NARQPDDIEYTSLTCAALLLSFAVGSSADIEQ 346

tr Q6TXD9 **Nucleocapsid protein [Vesicular stomatitis virus]** 422 AA  
Q6TXD9\_9RHAB

align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 13/32 (40%), Positives = 21/32 (65%)

Query: 78 NSDEPDTIEYCNLGCRSSVCDYMNAAADDEE 109  
N+ +PD IEY +L C S + + V ++AD E+  
Sbjct: 315 NARQPDDIEYTSLTCAALLLSFAVGSSADIEQ 346

tr Q9CA14 **Putative phorbol ester / diacylglycerol binding** 1506  
Q9CA14\_ARATH **protein;** AA  
61157-67783 [T32E8.13] [Arabidopsis thaliana (Mouse-ear align  
cress)]

Score = 31.2 bits (69), Expect = 3.7  
Identities = 31/121 (25%), Positives = 45/121 (36%), Gaps = 28/121 (23%)

Query: 31 CRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSG-----LSCPK--GFPKLALESNSD 80  
C+ T+ + CY L G LCS C+L +G L CPK G K L S ++  
Sbjct: 301 CKATVHKKCYGLLED SGKPWLCSW---CELENGRADSERPCLLCPPKGGIILKPVL-SKTE 356

Query: 81 EPDTIEYCNLGCRSSVCDYMN-----AAADDEEMKLYVENC---GDACVNFCN 126  
E+ +L C + + + + KL C AC+ CN  
Sbjct: 357 NGGPAEFAHLFCSLWMPEVYIEDLKKMEPIILNFPGIKETRRKLLCNLCKVKSGACIRCCN 416

Query: 127 G 127  
G  
Sbjct: 417 G 417

tr Q620M9 **Hypothetical protein CBG02727 [CBG02727]** 1360  
Q620M9\_CAEBR **[Caenorhabditis briggsae]** AA  
align

Score = 31.2 bits (69), Expect = 3.7  
Identities = 26/98 (26%), Positives = 35/98 (35%), Gaps = 19/98 (19%)

Query: 38 NCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTIEY-CNLGCRSSV 96  
+C LC+S Q + C+ G + N P T+ Y CNL C S  
Sbjct: 1241 DCQGLCKSNSPQCIQGCDASCQQLCGTA-----PNPAVPLTVNINC�LP CDSQC 1289

Query: 97 CDYMN-----AAADDEEMKLYVENCGDACVNFCNG 127  
+ A A E + V +C DAC C G  
Sbjct: 1290 TQQCYHQAPT CAPACAQACEAQCPVVSCEDACQTVCKG 1327

tr Q84BD4 **CHP [Myxococcus xanthus]** 300 AA  
Q84BD4\_MYXXA align

Score = 30.8 bits (68), Expect = 4.8

Identities = 22/63 (34%), Positives = 26/63 (40%), Gaps = 7/63 (11%)

Query: 77 SNSDEPDTIEYCN-LGCRS--SVC----DYMVNAAADDEEMKLYVENCGDACVNFCNGDA 129  
S SD P T + C LG S S+C D + N D E L N + CV C D

Sbjct: 58 SGSDCPSTAKTCAPLGGTSTTSICQCSTDVLCNGGTDSESTGLVCSNLDNVCVTACTSDT 117

Query: 130 GLT 132

T

Sbjct: 118 ECT 120

tr Q69JY6 **Putative thaumatin-like protein [P0569E11.21] [Oryza** 312  
Q69JY6\_ORYSA **sativa** AA  
(japonica cultivar-group)] align

Score = 30.8 bits (68), Expect = 4.8

Identities = 29/111 (26%), Positives = 44/111 (39%), Gaps = 7/111 (6%)

Query: 26 EGKSCCRTTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGFPKLALESNSDEPDTI 85  
+GK C T + CR GA +T+ L SG K + ++L + P +

Sbjct: 93 DGKGT CATGDCGSGEVECRGAGATPP-ATLVEFTLGSGGGGGKDYDVSILVDGYNLPMVV 151

Query: 86 EYCNLGCRSSVCDYMVNAAADDEEMKLYVENCGDACVNF-----CNGDAG 130  
E GC ++ C +N E + + C AC F C+GD G

Sbjct: 152 EAAAAGCPATGCVVDLNRCPAELKAGHGQACRSACEAFGTPEYCCSGDHG 202

tr Q6PTE0 **Methionine adenosyltransferase (Fragment) [Monosiga** 322  
Q6PTE0\_MONBE **brevicollis]** AA  
align

Score = 30.8 bits (68), Expect = 4.8

Identities = 19/74 (25%), Positives = 32/74 (42%)

Query: 59 KLTSGLSCPKGFPKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADDEEMKLYVENCG 118  
+L G + PK + + + E T E R V + +V A D++ +++ G

Sbjct: 160 ELKD GATIPKRVTIVISTQHS EDTNEQLRKDLREKVINVVVPAELMDDKT V FHLQPSG 219

Query: 119 DACVNFCNGDAGLT 132

+ GDAGLT

Sbjct: 220 RFVIGGPQGDAGLT 233

tr Q6PVY8 **Zinc finger protein [Tranpr] [Oryza sativa (japonica** 302 AA  
Q6PVY8\_ORYSA **cultivar-group)]** align

Score = 30.4 bits (67), Expect = 6.3

Identities = 17/51 (33%), Positives = 21/51 (40%), Gaps = 3/51 (5%)

Query: 86 EYCNLCG---RSSVCDYVMNAAADDEEMKLYVENCGDACVNFCNGDAGLTS 133  
 E+C C SVCD DEE+ + CG+ V D G TS  
 Sbjct: 230 EHCQFACPLCSKSVCDMSKAWERLDEELATISDTGKNKMRILCNDGATS 280

tr Q6AVT3 Hypothetical protein OSJNBa0027J18.18 [OSJNBa0027J18.18] 77 AA  
 Q6AVT3\_ORYSA [Oryza  
 sativa (japonica cultivar-group)] align

Score = 30.4 bits (67), Expect = 6.3  
 Identities = 15/54 (27%), Positives = 24/54 (43%)

Query: 13 LLILGLVLEQVQVEGKSCCRTLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSLSC 66  
 LLI GLV+ + ++ C + Y C + ++L CRC G+ C  
 Sbjct: 12 LLISGLVMLERIEHTEAVCTLFCAKGTYYITCSNHPYEQLYGCACRCAPPDGVDC 65

tr Q95RQ1 LD16414p (CG7447-PA, isoform A) (Cg7447-pb, isoform b) 512  
 Q95RQ1\_DROME [CG7447] AA  
 [Drosophila melanogaster (Fruit fly)] align

Score = 30.4 bits (67), Expect = 6.3  
 Identities = 22/80 (27%), Positives = 31/80 (38%), Gaps = 10/80 (12%)

Query: 51 LCSTVCR----CKLTSGLSLCPKGFPKLALESNSDEPDTIEYCNLCGRSSVCDYMVNA--- 103  
 +CS C+ C S SCP GF E + DE T + C+ C ++ Y  
 Sbjct: 331 ICSARCQNGGNTAPSTCSCPTGFTGRFCEQDVDECQTEKPCDQQCINTHGSYFCRCRQG 390

Query: 104 ---AADDEEMKLYVENCGDA 120  
 +D + K N DA  
 Sbjct: 391 FVLQSDQQSCKKVSTNADDA 410

tr Q7PM75 ENSANGP00000010141 (Fragment) [ENSANGG00000007652] 320  
 Q7PM75\_ANOGA [Anopheles  
 gambiae str. PEST] AA  
align

Score = 30.4 bits (67), Expect = 6.3  
 Identities = 18/45 (40%), Positives = 22/45 (48%), Gaps = 9/45 (20%)

Query: 31 CRTTLGR-----NCYNLCRSRG-AQKLCSTV--CRCKLTSGLSLSC 66  
 C T GR NC LC RG K+ T+ CRC+ T+G C  
 Sbjct: 259 CSVTRGRRLHPDNCATLCCGRGYTTKVIKTLEKCRCRFTNGRCC 303

sp P79847 Lysozyme C precursor (EC 3.2.1.17) (1,4-beta-N- 148  
 LYSC\_PYGNE acetylmuramidase C) AA  
 [LYZ] [Pygathrix nemaeus (Dove langur)] align

Score = 30.0 bits (66), Expect = 8.2

Identities = 30/112 (26%), Positives = 43/112 (37%), Gaps = 16/112 (14%)

```
Query: 10  MVCLLILGLVLEQVQVEGKSCCRTTLGRNCYNL-CRSGAQKLCSTVCRCKLTSGLSCP 68
           M L+ILGLVL V V+GK R L R L L + VC K SG +
Sbjct: 1   MKALIILGLVLLSVTVQGIKIFERCELARTLKKLGLDGYKGVSLANWVCLAKWESGYNTEA 60

Query: 69  GFPKLALES-----NSDEPDTIEYCNLGCRSSVCDYMNAAA 105
           ES N P ++ C++ C + + + + +A A
Sbjct: 61  TNYNPGDESTDYGIFQINSRYWCNNGKTPGAVDACHISCSALLQNNIADAVA 112
```

Database: EXPASY/UniProt

Posted date: Feb 16, 2005 2:07 PM

Number of letters in database: 574,459,479

Number of sequences in database: 1,794,555

| Lambda | K     | H     |
|--------|-------|-------|
| 0.321  | 0.137 | 0.435 |

Gapped

| Lambda | K      | H     |
|--------|--------|-------|
| 0.267  | 0.0410 | 0.140 |

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

length of query: 136

length of database: 574,459,479

effective HSP length: 112

effective length of query: 24

effective length of database: 373,469,319

effective search space: 8963263656

effective search space used: 8963263656

T: 11

A: 40

X1: 16 ( 7.4 bits)

X2: 38 (14.6 bits)

X3: 64 (24.7 bits)

S1: 41 (21.9 bits)

S2: 66 (30.0 bits)

Wallclock time: 4 seconds

|                                                                                                                      |                          |                               |                            |                                  |                            |
|----------------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------|----------------------------|----------------------------------|----------------------------|
|  <a href="#">ExPASy Home page</a> | <a href="#">Site Map</a> | <a href="#">Search ExPASy</a> | <a href="#">Contact us</a> | <a href="#">Proteomics tools</a> | <a href="#">Swiss-Prot</a> |
|----------------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------|----------------------------|----------------------------------|----------------------------|



YSTE:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1951-2005/Feb W3

(c) format only 2005 The Dialog Corp.

**\*File 155: Medline has been reloaded; accession numbers have changed.**

Please see HELP NEWS 154.

File 654:US Pat.Full. 1976-2005/Feb '24

(c) Format only 2005 The Dialog Corp.

File 399:CA SEARCH(R) 1967-2005/UD=14209

(c) 2005 American Chemical Society

**\*File 399: Use is subject to the terms of your user/customer agreement.**

Alert feature enhanced for multiple files, etc. See HELP ALERT.

File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210

(c) 2005 WIPO/Univentio

File 340:CLAIMS(R)/US Patent 1950-05/Feb 24

(c) 2005 IFI/CLAIMS(R)

**\*File 340: 2004 Reload is online as of October 6, 2004. Pricing changes effective October 1, 2004. See HELP NEWS 340 for details.**

File 5:Biosis Previews(R) 1969-2005/Feb W3

(c) 2005 BIOSIS

**\*File 5: Price change effective Jan 1, 2005. Enter HELP**

RATES 5 for details.

File 348:EUROPEAN PATENTS 1978-2005/Feb W03

(c) 2005 European Patent Office

File 347:JAPIO Nov 1976-2004/Oct(Updated 050208)

(c) 2005 JPO & JAPIO

**\*File 347: JAPIO data problems with year 2000 records are now fixed.**

Alerts have been run. See HELP NEWS 347 for details.

File 65:Inside Conferences 1993-2005/Feb W3

(c) 2005 BLDSC all rts. reserv.

File 35:Disertation Abs Online 1861-2005/Feb

(c) 2005 ProQuest Info&Learning

File 342:Derwent Patents Citation Indx 1978-05/200510

(c) 2005 Thomson Derwent

File 203:AGRIIS 1974-2004/Nov

Dist by NAL, Intl Copr. All rights reserved

File 156:ToxFile 1965-2005/Feb W3

(c) format only 2005 The Dialog Corporation

**\*File 156: Updating of ToxFile has resumed, with UD=20041205.**

File 94:JICST-EPlus 1985-2005/Jan W2

(c)2005 Japan Science and Tech Corp(JST)

File 398:Chemsearch 1957-2005/Jan

(c) 2005 Amer.Chem.Soc.

**\*File 398: Use is subject to the terms of your user/customer agreement.**

Problems with SORT. RANK charge added. See HELP RATES 398.

File 357:Derwent Biotech Res. 1982-2005/Feb W4

(c) 2005 Thomson Derwent & ISI

File 73:EMBASE 1974-2005/Feb W3

(c) 2005 Elsevier Science B.V.

**\*File 73: Price change effective Jan 1, 2005. Enter HELP**

RATES 73 for details.

File 50:CAB Abstracts 1972-2005/Jan

(c) 2005 CAB International

Set Items Description

--- -----

Executing TD918

>>>SET HILIGHT: use ON, OFF, or 1-5 characters

5311447 ALPHA

15646 THIONIN?

S1 373 ALPHA (2N) THIONIN?

?rd

>>>Duplicate detection is not supported for File 654.

>>>Duplicate detection is not supported for File 349.

>>>Duplicate detection is not supported for File 340.

>>>Duplicate detection is not supported for File 348.

>>>Duplicate detection is not supported for File 347.

>>>Duplicate detection is not supported for File 342.  
>>>Duplicate detection is not supported for File 398.

>>>Records from unsupported files will be retained in the RD set.

...examined 50 records (50)  
...examined 50 records (100)  
...examined 50 records (150)  
...examined 50 records (200)  
...examined 50 records (250)  
...examined 50 records (300)  
...examined 50 records (350)  
...completed examining records  
S2 313 RD (unique items)  
?e edta

| Ref | Items  | RT | Index-term                                     |
|-----|--------|----|------------------------------------------------|
| E1  | 279736 | 7  | *EDTA                                          |
| E2  | 1      |    | EDTA (3-)                                      |
| E3  | 1      |    | EDTA + FERRIC CITRATE                          |
| E4  | 1      |    | EDTA & GROWTH & REPRODUCTION                   |
| E5  | 1      |    | EDTA ABRASION                                  |
| E6  | 1      |    | EDTA ABSORPTION ION EXCHANGE                   |
| E7  | 1      |    | EDTA ACETIC ACID CALCIUM CHLORIDE HYDROGEN PER |
| E8  | 1      |    | EDTA ACID CITRATE DEXTROSE SODIUM CITRATE HEPA |
| E9  | 1      |    | EDTA ACTION                                    |
| E10 | 2      |    | EDTA ADDITION                                  |
| E11 | 1      |    | EDTA ADP REDUCTION AGGREGATION                 |
| E12 | 1      |    | EDTA ADP-RIBOSYLTRANSFERASE                    |

Enter P or PAGE for more

?s e1 or e2 or e9 or e10

279736 EDTA  
1 EDTA (3-)  
1 EDTA ACTION  
2 EDTA ADDITION

S3 279736 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'

?p

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E13 | 1     | EDTA ALGINIC-ACID HUMIC-ACID NITRILOTRIACETIC- |
| E14 | 1     | EDTA ALPHA AMYLASE ACTIVITY AMINO-ACIDS/       |
| E15 | 1     | EDTA ALPHA AMYLASE AMMONIA CITRIC BUFFER AGAR  |
| E16 | 1     | EDTA AMINOCHROME NORADRENOCROME                |
| E17 | 1     | EDTA AMMONIUM ACETATE AMMONIUM OXALATE SODIUM  |
| E18 | 1     | EDTA AMMONIUM ACETATE PH ORGANIC MATTER        |
| E19 | 1     | EDTA AMMONIUM CARBONATE AMMONIUM ACETATE DITHI |
| E20 | 1     | EDTA AMMONIUM CARBONATE AMMONIUM ACETATE NUTRI |
| E21 | 1     | EDTA AMMONIUM CHLORIDE SUN LIGHT INSOLATION TE |
| E22 | 1     | EDTA AMMONIUM COMPLEXES                        |
| E23 | 1     | EDTA AMMONIUM HYDROXIDE SODIUM CHLORIDE BEHAVI |
| E24 | 1     | EDTA AMMONIUM ION POLLUTION INDICATOR GROWTH I |

Enter P or PAGE for more

?p

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E25 | 1     | EDTA AMMONIUM SALT                             |
| E26 | 1     | EDTA AMP MAGNESIUM                             |
| E27 | 1     | EDTA AMP MANGANESE ADENYLATE KINASE            |
| E28 | 1     | EDTA AMPLIFIED CELL DEPLETION FOCAL ATYPIAS PR |
| E29 | 1     | EDTA AMYLAMINE SALT                            |
| E30 | 1     | EDTA ANALOG                                    |
| E31 | 1     | EDTA ANALYSIS                                  |
| E32 | 1     | EDTA AND ASCORBIC ACID ON IRON ABSORPTION      |
| E33 | 1     | EDTA AND DTPA                                  |
| E34 | 1     | EDTA AND MAGNESIUM                             |
| E35 | 1     | EDTA AND SOMATOTROPIN AFFECT CALCAEMIC AND PAR |

E36 1 EDTA ANILINE HYDROXYLATION AMINOPYRINE N DEMET

Enter P or PAGE for more

?p

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E37 | 1     | EDTA ANTI COAGULATION                          |
| E38 | 1     | EDTA ANTIBODY INTESTINAL CALCIUM BINDING PROTE |
| E39 | 1     | EDTA ANTICOAGULANT                             |
| E40 | 1     | EDTA ANTICOAGULANT AGENT BLOOD COLLECTION METH |
| E41 | 2     | EDTA ANTICOAGULATION                           |
| E42 | 1     | EDTA ANTIDOTE HEMO GLOBIN CASEIN FIBRINOGEN CO |
| E43 | 1     | EDTA ANTIDOTE METABOLIC-DRUG                   |
| E44 | 1     | EDTA ANTIDOTE METABOLIC-DRUG GLUTATHIONE HEMO  |
| E45 | 1     | EDTA ANTIDOTE METABOLIC-DRUG METAL TOXICITY    |
| E46 | 1     | EDTA ANTIDOTE METABOLIC-DRUG PHARMACO KINETICS |
| E47 | 1     | EDTA ANTIDOTE-DRUG                             |
| E48 | 1     | EDTA ANTIDOTE-DRUG CHELATING AGENT USE PEDIATR |

Enter P or PAGE for more

?p

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E49 | 1     | EDTA ANTIDOTE-DRUG EGYPTIAN POLYVALENT ANTI VE |
| E50 | 1     | EDTA ANTIDOTE-DRUG INCREASED ZINC LEVELS LOWER |

?e e1

| Ref | Items | Type | RT | Index-term                      |
|-----|-------|------|----|---------------------------------|
| R1  | 53755 |      | 7  | *EDTA                           |
| R2  | 35601 | X    | 66 | EDETIC ACID                     |
| R3  | 0     | F    | 1  | ETHYLENEDIAMINETETRAACETIC ACID |
| R4  | 1146  | B    | 10 | ANTICOAGULANTS                  |
| R5  | 989   | B    | 11 | CHELATING AGENTS                |

?s r1

S4 53755 'EDTA'

?e r3

| Ref | Items | Type | RT | Index-term                          |
|-----|-------|------|----|-------------------------------------|
| R1  | 12    |      | 5  | *ETHYLENEDIAMINETETRAACETIC ACID    |
| R2  | 0     | S    | 3  | (ETHYLENEDINITRILO)TETRAACETIC ACID |
| R3  | 35601 | X    | 65 | EDETIC ACID                         |
| R4  | 7584  | U    | 4  | EDTA                                |

?s r1-r4

12 ETHYLENEDIAMINETETRAACETIC ACID  
0 (ETHYLENEDINITRILO)TETRAACETIC ACID  
35601 EDETIC ACID  
7584 EDTA  
S5 44607 R1-R4

?ds

| Set | Items  | Description                                               |
|-----|--------|-----------------------------------------------------------|
| S1  | 373    | ALPHA (2N) THIONIN?                                       |
| S2  | 313    | RD (unique items)                                         |
| S3  | 279736 | 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION' |
| S4  | 53755  | 'EDTA'                                                    |
| S5  | 44607  | R1-R4                                                     |

?s s2 and (s3 or s4 or s5 or edta? or ethylenediamine?)

313 S2  
279736 S3  
53755 S4  
44607 S5  
280289 EDTA?  
198685 ETHYLENEDIAMINE?

S6 79 S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)

?pause

?s s6/2002:2005

>>>One or more prefixes are unsupported

>>> or undefined in one or more files.  
>>>Year ranges not supported in one or more files  
79 S6  
15341873 PY=2002 : PY=2005  
S7 51 S6/2002:2005  
?s s6 not s7  
79 S6  
51 S7  
S8 28 S6 NOT S7  
?t s8/6,kwic/all  
>>>KWIC option is not available in file(s): 398, 399

**8/6,KWIC/1 (Item 1 from file: 654)**  
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4569339 \*\*IMAGE Available  
Derwent Accession: 1995-269272

**Utility**

**C/ Treatment for juvenile diabetes**

Fulltext Word Count: 5801  
Number of Claims: 15  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 5  
Number of Figures: 9  
Number of US cited patent references: 2  
Number of non-US cited patent references: 1  
Number of non-patent cited references: 27

**Description of the Invention:**

...Y. The TFG[alpha] transgenic line MT-42 used, which expresses high levels of TFG[ **alpha** ] from a **metallothionine** promoter, is described in Jhappan et al, Cell, 61:1137-1146 (1990...CsCl gradient purification, and dialyzed extensively against injection buffer (5 mM NaCl; 0.1 mM **EDTA** ; 5 mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms...

**8/6,KWIC/2 (Item 2 from file: 654)**  
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4525836 \*\*IMAGE Available  
Derwent Accession: 2001-407325

**Utility**

**C/ Trabecular meshwork induced glucocorticoid response (TIGR) fusion protein**  
**; POLYPEPTIDE ASSOCIATED WITH PROPER EYE FUNCTION; FOR THE DIAGNOSIS OF VISION DEFECTS**

Fulltext Word Count: 13759  
Number of Claims: 8  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 4  
Number of Figures: 4  
Number of US cited patent references: 11  
Number of non-US cited patent references: 6  
Number of non-patent cited references: 36

**Description of the Invention:**

...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

4461438 \*\*IMAGE Available

Derwent Accession: 1996-230600

Utility

C/ Neutralization of food allergens by thioredoxin

; CONTACTING PROTEIN WITH AMOUNT OF THIOREDOXIN, NICOTINAMIDE ADENINE  
DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE AND NADPH OR AMOUNT OF  
THIOREDOXIN AND DITHIOTHREITOL EFFECTIVE FOR DECREASING ALLERGENICITY OF  
PROTEIN; ADMINISTERING

Fulltext Word Count: 29804

Number of Claims: 5

Exemplary or Independent Claim Number(s): 3

Number of Drawing Sheets: 6

Number of Figures: 6

Number of US cited patent references: 3

Number of non-US cited patent references: 3

Number of non-patent cited references: 60

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:

... **Purothionin** [ **alpha** ] from bread wheat and **purothionins** [ **alpha** ]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

#### Utility

C/ Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically, proliferating cells or to upregulate nitrosative stress defenses

; ALPHA-ALKYL-S-ALKYL-HOMOCYSTEINE SULFOXIMINES WHEREIN ALPHA-ALKYL CONTAINS 2 TO 8 CARBON ATOMS AND S-ALKYL CONTAINS 1 TO 10 CARBON ATOMS EXCEPT FOR ALPHA-ETHYL-METHIONINE SULFOXIMINE

Fulltext Word Count: 28487

Number of Claims: 15

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 2

Number of Figures: 2

Number of US cited patent references: 28

Number of non-US cited patent references: 1

Number of non-patent cited references: 30

#### Summary of the Invention:

...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [ **alpha** ]-ethyl- **buthionine** sulfoximine, [ **alpha** ]-propyl- **buthionine** sulfoximine, [ **alpha** ]-isopropyl- **buthionine** sulfoximine, and [ **alpha** ]-tert butyl- **buthionine** sulfoximine...

#### Description of the Invention:

...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [ **alpha** ]-ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acid...

...Other sulfoximines embraced by the genus described above, include, for example, [ **alpha** ]-propyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-isopropyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-tert butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [alpha]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also... where the [alpha]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl<sub>2</sub>, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[alpha]-aminobutyrate (an L-cysteine analog), 0.3... 2, 142 mM KCl, 36 mM MgCl<sub>2</sub>, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed...

...inactivation in 30 min. A similar level of inactivation was achieved with 2 mM with [ **alpha** ]-ethyl-DL- **buthionine** -SR-sulfoximine (DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [ **alpha** ]-ethyl-DL- **buthionine** -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma...

...protocol similar to that described here for the E. coli enzyme, it was found that [ **alpha** ]-ethyl-DL- **buthionine** -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [ **alpha** ]-ethyl-L-

**buthionine** -S-sulfoximine, [ **alpha** ]-ethyl-BSO has about 0.1% the inhibitory activity of L-buthionine-S-sulfoximine with...A patient with *E. coli* caused gastroenteritis with bloody diarrhea is administered [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...

...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):

...3. A sulfoximine as claimed in claim 2 which is [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine...

...A sulfoximine as claimed in claim 1 which is selected from the group consisting of [ **alpha** ]-ethyl- **buthionine** sulfoximine, [ **alpha** ]-propyl- **buthionine** sulfoximine, [ **alpha** ]-isopropyl- **buthionine** sulfoximine, [ **alpha** ]-butyl- **buthionine** sulfoximine, and [ **alpha** ]-tert butyl- **buthionine** sulfoximine...

8/6,KWIC/5 (Item 5 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4417299

Derwent Accession: 2001-060016

#### Utility

C/ Methods for the diagnosis of glaucoma

; NUCLEIC ACID HAVING DEFINED NUCLEOTIDE SEQUENCE WHICH CODES FOR TRABECULAR MESHWORK INDUCED GLUCOCORTICOID RESPONSE PROTEIN

Fulltext Word Count: 13915

Number of Claims: 14

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 4

Number of Figures: 4

Number of US cited patent references: 19

Number of non-US cited patent references: 9

Number of non-patent cited references: 35

#### Description of the Invention:

...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as **EDTA** ; and sugar ... pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and

8/6,KWIC/6 (Item 6 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4413998

Derwent Accession: 1992-415779

#### Utility

C/ Antipathogenic peptides and compositions containing same

; NUCLEOTIDE SEQUENCES CODING AN AMINO ACID SEQUENCE ASSOCIATED WITH THE PREVENTION OF INFECTION BY FUNGAL AND BACTERIAL PLANT PARASITES

Fulltext Word Count: 14201

Number of Claims: 23

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 1

Number of Figures: 1

Number of US cited patent references: 2

Number of non-patent cited references: 10

#### Description of the Invention:

...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA**, pH 7.5...For control purposes two standards are used in the above test that is [ **alpha** ]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...is maintained on a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco # 500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/6,KWIC/7 (Item 7 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4377970

Derwent Accession: 1993-152468

#### Utility

C/ Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins

Fulltext Word Count: 26012

Number of Claims: 7

Exemplary or Independent Claim Number(s): 7

Number of Drawing Sheets: 53

Number of Figures: 63

Number of US cited patent references: 3

Number of non-US cited patent references: 3

Number of non-patent cited references: 44

#### Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

#### Description of the Drawings:

...FIG. 5 is a graph showing the effect of **purothionin** [ **alpha** ] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

#### Description of the Invention:

...Purothionin a from bread wheat and **purothionins** [ **alpha** ]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124: 237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours.



The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/8 (Item 8 from file: 654)

DIALOG(R) File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4377417

Derwent Accession: 1993-152468

#### Utility

C/ Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins

Fulltext Word Count: 28198

Number of Claims: 43

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 53

Number of Figures: 63

Number of US cited patent references: 3

Number of non-US cited patent references: 3

Number of non-patent cited references: 52

#### Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area,

#### Description of the Drawings:

...FIG. 5 is a graph showing the effect of **purothionin** [ **alpha** ] and CM-1 [ **alpha** ]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

#### Description of the Invention:

... **Purothionin** [ **alpha** ] from bread wheat and **purothionins** [ **alpha** ]-1 and [ **beta** ] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [ **beta** ] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...reaction was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing mM **EDTA** and 16% glycerol in a final volume of ...5, conditions were as in FIG. 4 except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [  $\mu$ g ] or CM-1, 20 [  $\mu$ g ] was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [ **beta** ] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure (FIG. 7...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and

NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/9 (Item 9 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4331037

Derwent Accession: 1998-482938

**Utility**

C/ **Production process of cross-linked polyaspartic acid resin  
; HYDROLYSING A CROSSLINKED POLYSUCCINIMIDE**

Fulltext Word Count: 35157

Number of Claims: 15

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 1

**Description of the Invention:**

...be mentioned. Their specific examples can include polyamines, for example, aliphatic polyamines such as hydrazine, **ethylenediamine**, propylenediamine, 1,4-butanediamine, pentamethylenediamine, hexamethylenediamine, heptamethylenediamine, octamethylenediamine, nonamethylenediamine, decamethylenediamine, undecamethylenediamine, dodecamethylenediamine, tetradecamethylenediamine, hexadecamethylenediamine, 1...diamino-7-hydroxyazelaic acid, isolysine, 3,5-diaminohexanoic acid, [alpha],[gamma]-diaminobutyric acid, djenkolic acid, **cystathionine**, cystine disulfoxide, [ **alpha** ],[epsilon]-diamino-[beta]-hydropimelic acid, hypusine, [gamma]-hydroxyornithine, [alpha]-hydroxylysine, lanthionine, lysinonorleucine, lysovitoxine, and loseanine...are those having less odor and high reactivity with imide rings in polysuccinimide, that is, **ethylenediamine**, propylenediamine, 1,4-butanediamine, heptamethylenediamine, hexamethylenediamine, and cystamine. In addition, protein-constituting amino acids such...

8/6,KWIC/10 (Item 10 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4314235

Derwent Accession: 1998-216927

**Utility**

C/ **Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically proliferating cells or to upregulate nitrosative stress defenses**

Fulltext Word Count: 30970

Number of Claims: 66

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 2

Number of Figures: 2

Number of US cited patent references: 25

Number of non-US cited patent references: 1

Number of non-patent cited references: 34

**Summary of the Invention:**

...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [ **alpha** ]-ethyl- **buthionine** sulfoximine, [ **alpha**

] -propyl- **buthionine** sulfoximine, [ **alpha** ] -isopropyl- **buthionine** sulfoximine, and [ **alpha** ] -tert butyl- **buthionine** sulfoximine

Description of the Invention:

...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [ **alpha** ] -ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acid. Other sulfoximines embraced by the genus described above, include, for example, [ **alpha** ] -propyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ] -isopropyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ] -butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ] -tert butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ] -ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [alpha]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also...where the [alpha]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl<sub>2</sub>, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[alpha]-aminobutyrate (an L-cysteine analog), 0.3...2, 142 mM KCl, 36 mM MgCl<sub>2</sub>, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed similar level of inactivation was achieved with 2 mM with [ **alpha** ] -ethyl-DL- **buthionine** -SR-sulfoximine (DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...

...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [ **alpha** ] -ethyl-DL- **buthionine** -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma ...

...protocol similar to that described here for the E. coli enzyme, it was found that [ **alpha** ] -ethyl-DL- **buthionine** -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine, [ **alpha** ] -ethyl-BSO has about A patient with E. coli caused gastroenteritis with bloody diarrhea is administered [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...

...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):

...7. The method of claim 6 wherein the manipulator of nitrosative stress is [ **alpha** ] -ethyl-L- **buthionine** -S-sulfoximine...

8/6, KWIC/11 (Item 11 from file: 654)  
DIALOG(R) File 654: (c) Format only 2005 The Dialog Corp. All rts. reserv.

4272687

Derwent Accession: 1996-497351

Utility

C/ S-adenosyl methionine regulation of metabolic pathways and its use in diagnosis and therapy

Fulltext Word Count: 35397

Number of Claims: 18

Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 12  
Number of Figures: 12  
Number of US cited patent references: 10  
Number of non-US cited patent references: 2  
Number of non-patent cited references: 38

Description of the Invention:

...buffer (15 mM KCl, 60 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 10% glycerol; to this was added DTT to make 1 mM and a protease mix...buffer (15 mM KCl, 60 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) by gentle mixing for 5 minutes followed by centrifugation for 60 second at 1000Xg. Buffer A (250 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) in a volume of 0.5 ml, is added to the affinity...

...successively with Buffer B (750 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) and Buffer C (7M urea, 50 mM Hepes, pH 7.5, 1 mM **EDTA** , 1 mM DTT) to yield "supernatant B" and "supernatant C". The protein concentrations of Wash...

Non-exemplary or Dependent Claim(s):

...for Pathway 1, S-adenosylmethionine (SAM), S-adenosyl homocysteine (SAH), adenosine, homocysteine, glutathione disulfide, **cystathionine** , [ **alpha** ]-ketobutyrate, cysteine, cystine, taurine, choline, betaine, dimethylglycine, methylglycine, glycine, serine, folate, tetrahydrofolate, methylene tetrahydrofolate, methyltetrahydrofolate...

8/6,KWIC/12 (Item 12 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4244044 \*\*IMAGE Available

Derwent Accession: 1998-086960

Utility

C/ Full length transcript (FLt) promoter from figwort mosaic caulimovirus (FMV) and use to express chimeric genes in plant cells

Fulltext Word Count: 12562

Number of Claims: 12

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 17

Number of Figures: 11

Number of US cited patent references: 10

Number of non-US cited patent references: 1

Number of non-patent cited references: 18

Description of the Invention:

...extraction buffer (50 mM NaPO<sub>4</sub>, pH 7.0, 10 mM [beta]-mercaptoethanol, 10 mM Na<sub>2</sub> **EDTA** , 0.8% Na Sarkosyl, 0.1% Triton X-100), and centrifuged for 10 min. at full...extraction buffer (50 mM NaPO<sub>4</sub>, pH 7.0, 10 mM [beta]-mercaptoethanol, 10 mM Na<sub>2</sub> **EDTA** , 0.1% Na Sarkosyl, 0.1% Triton X-100), and centrifuged for 10 min at...5 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], 0.5 mM K<sub>4</sub>[Fe(CN)<sub>6</sub>], 10 mM **EDTA** , 1 mg/ml 5-bromo-4-chloro-3-indolyl-[beta]-D-glucuronide (X-gluc) in...5. Bacterial or nematode pathogen resistance, (developed with [ **alpha** ]- hordothionin gene, Bt toxin gene, beet cyst nematode resistant locus...

8/6,KWIC/13 (Item 13 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4198164

Derwent Accession: 1999-288106

Utility

C/ Increasing the digestibility of food proteins by thioredoxin reduction

**; FOOD TREATMENT WITH THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE  
PHOSPHATE-THIOREDOXIN REDUCTASE, REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE  
PHOSPHATE (NADPH) AND ADMINISTERING TREATED FOOD TO ANIMAL**

Fulltext Word Count: 35406

Number of Claims: 6

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 9

Number of Figures: 18

Number of US cited patent references: 3

Number of non-US cited patent references: 2

Number of non-patent cited references: 51

**Summary of the Invention:**

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

**Description of the Invention:**

... **Purothionin** [ **alpha** ] from bread wheat and **purothionins** [ **alpha** ]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume ... same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...with 30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/14 (Item 14 from file: 654)

DIALOG(R) File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4125212

Derwent Accession: 1995-269272

**Utility**

**C/ Treatment for diabetes using a gastrin/CCK receptor ligand and an EGF receptor ligand**

**; ADMINISTERING GASTRIN/CHOLECYSTOKININ RECEPTOR LIGAND AND EPIDERMAL GROWTH FACTOR RECEPTOR LIGAND TO EFFECT DIFFERENTIATION OF PANCREATIC ISLET PRECURSOR CELLS TO MATURE INSULIN SECRETING CELLS**

Fulltext Word Count: 5502  
Number of Claims: 9  
Exemplary or Independent Claim Number(s): 1,6  
Number of Drawing Sheets: 5  
Number of Figures: 9  
Number of non-patent cited references: 4

Description of the Invention:

...NY. The TGF[alpha] transgenic line MT-42 used, which expresses high levels of TGF[ alpha ] from a **metallothionine** promoter, is described in Jhappan et al, Cell, 61:1137-1146 (1990...CsCl gradient purification, and dialyzed extensively against injection buffer (5 mM NaCl; 0.1 mM **EDTA** ; 5 mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms...

**8/6,KWIC/15 (Item 15 from file: 654)**  
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4098317

**Utility**

C/ **Trabecular meshwork induced glucocorticoid response (TIGR) nucleic acid molecules**  
**; ACCURATE GLAUCOMA DIAGNOSIS**

Fulltext Word Count: 12136  
Number of Claims: 6  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 4  
Number of Figures: 4  
Number of US cited patent references: 10  
Number of non-US cited patent references: 6  
Number of non-patent cited references: 32

Description of the Invention:

...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

**8/6,KWIC/16 (Item 16 from file: 654)**  
DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4090242

Derwent Accession: 1999-095006

**Utility**

C/ **Methods for the diagnosis of glaucoma**

Fulltext Word Count: 13856  
Number of Claims: 10  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 4  
Number of Figures: 4  
Number of US cited patent references: 10  
Number of non-US cited patent references: 4  
Number of non-patent cited references: 30

Description of the Invention:

...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as **EDTA** ; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine** , **alpha** [sub]1 -acid glycoprotein, and TAT which were

maximally induced by one day dexamethasone treatment...

8/6,KWIC/17 (Item 17 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4022853

Derwent Accession: 1996-230600

#### Utility

C/ Neutralization of food allergens by thioredoxin

; DISULFIDE BONDS ARE REDUCED TO SULFHYDRYL DECREASING ALLERGENICITY OF FEEDS

Fulltext Word Count: 30535

Number of Claims: 25

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 6

Number of Figures: 6

Number of US cited patent references: 1

Number of non-patent cited references: 39

#### Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

#### Description of the Invention:

...Purothionin a from bread wheat and **purothionins** [ **alpha** ]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/18 (Item 18 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4019370

Derwent Accession: 1998-446069

**Utility**

**C/ Methods for the diagnosis of glaucoma  
; USING A GLUCOCORTICOID INDUCED PROTEIN**

Fulltext Word Count: 18029

Number of Claims: 106

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 4

Number of Figures: 4

Number of US cited patent references: 10

Number of non-US cited patent references: 5

Number of non-patent cited references: 31

**Description of the Invention:**

...other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as **EDTA**; and sugar alcohols such as mannitol or sorbitol...pattern of induction in HTM cells was distinguishable from other steroid induced proteins such as **metallothionine**, **alpha** [sub]1 -acid glycoprotein, and TAT which were maximally induced by one day dexamethasone treatment...

**8/6,KWIC/19 (Item 19 from file: 654)**

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

4003403

Derwent Accession: 1995-090847

**Utility**

**C/ Antimicrobial proteins from Allium  
; FUNGICIDES; BACTERICIDES**

Fulltext Word Count: 7927

Number of Claims: 11

Exemplary or Independent Claim Number(s): 1,2,6,8,10

Number of Drawing Sheets: 7

Number of Figures: 5

Number of non-US cited patent references: 2

**Summary of the Invention:**

...Rhizoctonia solani (Logemann et al, 1992, Biotechnol, 10:305-308); transgenic tobacco expressing a barley [ **alpha** ]- **thionin** has increased resistance to Pseudomonas bacterial pathogens (Carmona et al, 1993, Plant J, 3(3...)

**Description of the Invention:**

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 2 mM thiourea. After extraction, the slurry was mixed in a WARING blender and ...sample buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...filtration over a Chromaspin+TE-100 (Clontech) column equilibrated in 10 mM Tris, 1 mM **EDTA**, 300 mM NaCl, 0.05% (w/v) SDS (pH 8). RNA was subsequently removed by...

**8/6,KWIC/20 (Item 20 from file: 654)**

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv. .

3985641

Derwent Accession: 1998-059387

**Utility**

**C/ Polyimide precursor composition, method of forming polyimide film,  
electronic parts and liquid crystal element  
; COMPOSITION COMPRISING POLYAMIC ACID, CURE ACCELERATOR SELECTED FROM**



GROUP CONSISTING OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUND, AMINO ACID  
COMPOUND, AROMATIC COMPOUND HAVING TWO OR MORE HYDROXYL GROUPS

Fulltext Word Count: 18794

Number of Claims: 20

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 5

Number of Figures: 8

Number of US cited patent references: 2

Number of non-patent cited references: 4

Description of the Invention:

...pteridine, oxazole, benzoxazole, isoxazole, benzisoxazole, thiazole, benzothiazole, isothiazole, benzisothiazole, oxadiazole, thiadiazole, pyrroledione, isoindoledione, pyrrolidinedione, benzisoquinolinedione, **triethylenediamine**, and hexamethylenetetramine. These compounds may be in the form of an N-oxide compound...isoglutamine, [alpha]-methylglutamic acid, [beta]-hydroxyglutamic acid, [gamma]-hydroxyglutamic acid, [alpha]-aminoadipic acid, citrulline, lanthionine, **cystathionine**, phenylalanine, [ **alpha** ]-methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine, p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine, p-fluorophenylalanine, [beta]-(2...1,3-dione, N-hydroxypyrrolidine-2,5-dione, N-hydroxybenz de!isoquinoline-1,3-dione, **triethylenediamine**, hexamethylenetetramine, hydantoin, histidine, uracil, barbituric acid, dialuric acid, cytosine, anilinoacetic acid, N-(2-pyridyl)glycine...

Non-exemplary or Dependent Claim(s):

...cinnoline, naphthylidine, acridine, phenanthridine, benzoquinoline, benzisoquinoline, benzocinnoline, benzophthalazine, benzoquinoxaline, benzoquinazoline, phenanthroline, phenazine, carboline, perimidine, pteridine, **triethylenediamine**, and hexamethylenetetramine; or... isoglutamine, [alpha]-methylglutamic acid, [beta]-hydroxyglutamic acid, [gamma]-hydroxyglutamic acid, [alpha]-aminoadipic acid, citrulline, lanthionine, **cystathionine**, phenylalanine, [ **alpha** ]-methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine, p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine, p-fluorophenylalanine, [beta]-(2...

8/6,KWIC/21 (Item 21 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

3783044

Derwent Accession: 1992-150877

Utility

C/ Directed evolution of novel binding proteins  
; GENETIC PACKAGE, CHIMERIC

Fulltext Word Count: 112846

Number of Claims: 83

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 16

Number of Figures: 16

Number of US cited patent references: 19

Number of non-US cited patent references: 10

Number of non-patent cited references: 47

Description of the Invention:

...to the binding site. For a decapeptide with three isoenergetic conformations (e.g., [beta] strand, [ **alpha** ] helix, and reverse turn) at each residue, there are about 6.[multiplication dot]10[sup...

8/6,KWIC/22 (Item 22 from file: 654)

DIALOG(R)File 654:(c) Format only 2005 The Dialog Corp. All rts. reserv.

3644278

Derwent Accession: 1992-415779

**Utility**

C/ **Antipathogenic peptides and compositions containing the same**  
; **HERBICIDES**

Fulltext Word Count: 14350

Number of Claims: 8

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 1

Number of Figures: 1

Number of US cited patent references: 1

Number of non-US cited patent references: 1

Number of non-patent cited references: 8

**Summary of the Invention:**

...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...

**Description of the Invention:**

...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA**, pH 7.5...For control purposes two standards are used in the above test that is [ **alpha** ]1/[beta] **thionin** from wheat endosperm and LT26-thionin from barley leaves...medium is maintained on a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco #500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/6,KWIC/23 (Item 1 from file: 349)

DIALOG(R)File 349:(c) 2005 WIPO/Univentio. All rts. reserv.

00864262

**WHOLE CELL ENGINEERING BY MUTAGENIZING A SUBSTANTIAL PORTION OF A STARTING GENOME, COMBINING MUTATIONS, AND OPTIONALLY REPEATING**  
**INGENIERIE CELLULAIRE COMPLETE PAR MUTAGENESE D'UNE PARTIE SUBSTANTIELLE D'UN GENOME DE DEPART, PAR COMBINAISON DE MUTATIONS ET EVENTUELLEMENT REPETITION**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 336587

Publication Year: 2001

Fulltext Availability:

Detailed Description

**Detailed Description**

... kinase

23. Aleurone 1 75. CycIodextrin glycosyltransferase

24. Alpha hordodiinonin 76. Cyfindrical inclusion protein

25. **Alpha**-arnylase 77. **Cystathionine** synthase

26. Al@ha-hemogiobin 78. Delta- 1 2 desaturase

27. Aminoglycoside 3'-adenylyltransferase 79...methods, here, the interaction between biotin and avidin is overcome by employing denaturing conditions (fonnamide/ **EDTA**) to release the primer extension products of the sequencing reaction from the solid support for...

8/6,KWIC/24

(Item 2 from file: 349)

00144093

**HIGH LEVEL INDUCIBLE EXPRESSION OF HETEROLOGOUS GENES**

**EXPRESSION DE GENES HETEROLOGUES POUVANT ETRE INDUITE A UN HAUT NIVEAU**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 7195

Publication Year: 1988

Fulltext Availability:

Detailed Description

Claims

**Detailed Description**

... responsive transcription units have been identified including mouse mammary tumor virus (MMTV), mouse and human **metallothionins**, rat **alpha** 2u-globulin, and rat and human growth hormone, [See, e,g,, Hollenberg et al.

Nature...23M Tris@Cl pH 8.0) and the mixture held on ice for 5 min, **EDTA** (1 ml of .25 M **EDTA** pH 8.0) was added for an additional 5 min. on ice, and then 1...

**Claim**

... selected from the group consisting of the mouse mammary tumor virus LTR, mouse **metallothionine**, human **metallothionine**, rat **alpha** 2u-globulin, and rat and human growth hormone transcriptional control sequence and protions thereof which...

8/6,KWIC/25 (Item 1 from file: 340)

DIALOG(R)File 340:(c) 2005 IFI/CLAIMS(R). All rts. reserv.

2984640 9816693

**C/POLYIMIDE PRECURSOR COMPOSITION, METHOD OF FORMING POLYIMIDE FILM, ELECTRONIC PARTS AND LIQUID CRYSTAL ELEMENT; COMPOSITION COMPRISING POLYAMIC ACID, CURE ACCELERATOR SELECTED FROM GROUP CONSISTING OF NITROGEN-CONTAINING HETEROCYCLIC COMPOUND, AMINO ACID COMPOUND, AROMATIC COMPOUND HAVING TWO OR MORE HYDROXYL GROUPS**

Non-exemplary Claims: ...cinnoline, naphthylidine, acridine, phenanthridine, benzoquinoline, benzisoquinoline, benzocinnoline, benzophthalazine, benzoquinoxaline, benzoquinazoline, phenanthroline, phenazine, carboline, perimidine, pteridine, **triethylenediamine**, and hexamethylenetetramine; or the group consisting of pyridine, pyridazine, pyrimidine, pyrazine, triazine, tetrazine, oxazole, benzooxazole...

...isoglutamine, Alpha -methylglutamic acid, Beta -hydroxyglutamic acid, gamma -hydroxyglutamic acid, Alpha -aminoadipic acid, citrulline, **lanthionine**, **cystathionine**, phenylalanine, **Alpha** -methylphenylalanine, o-chlorophenylalanine, m-chlorophenylalanine, p-chlorophenylalanine, o-fluorophenylalanine, m-fluorophenylalanine, p-fluorophenylalanine, Beta -(2...

8/6,KWIC/26 (Item 1 from file: 348)

DIALOG(R)File 348:(c) 2005 European Patent Office. All rts. reserv.

01326487

**Treatment for diabetes**

**Behandlung von Diabetes**

**Traitement du diabete**

LANGUAGE (Publication,Procedural,Application): English; English; English

# FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | 200137 | 245        |
| SPEC A                             | (English) | 200137 | 5550       |
| Total word count - document A      |           |        | 5795       |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 5795       |

...SPECIFICATION NY. The TGF(alpha) transgenic line MT-42 used, which expresses high levels of TGF( **alpha** ) from a **metallothionine** promoter, is described in Jappan et al, Cell, 61:1137-1146 (1990).

INSGAS Transgene Construct...

...by CsCl gradient purification, and dialyzed extensively against injection buffer (5mM NaCl; 0.1 mM **EDTA** ; 5mM Tris-HCl pH 7.4). Fertilized oocytes from FVB inbred mice (Taconic Farms, Inc...

8/6,KWIC/27 (Item 2 from file: 348)

DIALOG(R)File 348:(c) 2005 European Patent Office. All rts. reserv.

00952289

Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods

Verwendung von Thiol-Redox-Proteinen zur Reduktion des intramolekularen Disulfid-Bindung bei Proteinen, zur Besserung der Qualität der Getreidenprodukte, des Te

Utilisation des proteines redox des groupes thiol pour la reduction des liaisonsdisulfide intramoleculaires, pour augmenter la qualite des produits cerealiers,

LANGUAGE (Publication,Procedural,Application): English; English; English

## FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | 9837   | 371        |
| SPEC A                             | (English) | 9837   | 25170      |
| Total word count - document A      |           |        | 25541      |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 25541      |

...SPECIFICATION measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin** ( **alpha** ) and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins

**Purothionin** ( **alpha** ) from bread wheat and **purothionins** ( **alpha** )-1 and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** ( **alpha** ) sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** ( **alpha** )-1 and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method Steps...

...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin** ( **alpha** ), 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the

type tested earlier, **purothionin** -( **alpha** ), failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124...

...240). However, in contrast to **purothionin**-a from bread wheat, two **purothionins** previously not examined ( **purothionins** ( **alpha** )-1 and (beta) from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the ( **alpha** )-type) by the SDS-PAGE fluorescence procedure (Fig. 7).

#### EXAMPLE 6

##### Quantitation of Reduction

The...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two ( **alpha** )-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30(degree)C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

##### CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/6,KWIC/28 (Item 3 from file: 348)  
DIALOG(R)File 348:(c) 2005 European Patent Office. All rts. reserv.

00709018

#### USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS

#### VERWENDUNG VON THIOLGRUPPEN ENTHALTENDEN REDOX-PROTEINEN ZUR REDUKTION VON DISULFIDBINDUNGEN

#### UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES

LANGUAGE (Publication,Procedural,Application): English; English; English

##### FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS B                           | (English) | 9901   | 992        |
| CLAIMS B                           | (German)  | 9901   | 1020       |
| CLAIMS B                           | (French)  | 9901   | 1208       |
| SPEC B                             | (English) | 9901   | 24864      |
| Total word count - document A      |           |        | 0          |
| Total word count - document B      |           |        | 28084      |
| Total word count - documents A + B |           |        | 28084      |

...SPECIFICATION measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin** ( **alpha** ) and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

##### Other proteins

**Purothionin** ( **alpha** ) from bread wheat and **purothionins** ( **alpha** )-1

and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin ( alpha )** sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin ( alpha )-1** and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

#### Routine Method Steps...

...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin ( alpha )**, 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin -( alpha )**, failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin -( alpha )** from bread wheat, two purothionins previously not examined ( **purothionins ( alpha )-1** and (beta) from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the ( **alpha** )-type) by the SDS-PAGE fluorescence procedure (Fig. 7).

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##### Quantitation of Reduction

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##### CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** .

Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

?logoff hold

26feb05 10:02:18 User228206 Session D2370.3

|         |                        |           |             |
|---------|------------------------|-----------|-------------|
| \$0.97  | 0.304                  | DialUnits | File155     |
| \$0.97  | Estimated cost File155 |           |             |
| \$7.21  | 1.222                  | DialUnits | File654     |
| \$5.50  | 22                     | Type(s)   | in Format 6 |
| \$5.50  | 22                     | Types     |             |
| \$12.71 | Estimated cost File654 |           |             |
| \$6.24  | 0.497                  | DialUnits | File399     |
| \$6.24  | Estimated cost File399 |           |             |
| \$1.22  | 0.257                  | DialUnits | File349     |
| \$0.50  | 2                      | Type(s)   | in Format 6 |
| \$0.50  | 2                      | Types     |             |
| \$1.72  | Estimated cost File349 |           |             |
| \$5.52  | 0.325                  | DialUnits | File340     |
| \$0.27  | 1                      | Type(s)   | in Format 6 |
| \$0.27  | 1                      | Types     |             |
| \$5.79  | Estimated cost File340 |           |             |
| \$1.88  | 0.328                  | DialUnits | File5       |
| \$1.88  | Estimated cost File5   |           |             |
| \$1.21  | 0.266                  | DialUnits | File348     |
| \$0.75  | 3                      | Type(s)   | in Format 6 |

\$0.75 3 Types  
 \$1.96 Estimated cost File348  
 \$1.18 0.108 DialUnits File347  
 \$1.18 Estimated cost File347  
 \$0.48 0.129 DialUnits File65  
 \$0.48 Estimated cost File65  
 \$0.23 0.056 DialUnits File35  
 \$0.23 Estimated cost File35  
 \$3.89 0.219 DialUnits File342  
 \$3.89 Estimated cost File342  
 \$0.15 0.061 DialUnits File203  
 \$0.15 Estimated cost File203  
 \$0.53 0.099 DialUnits File156  
 \$0.53 Estimated cost File156  
 \$0.31 0.088 DialUnits File94  
 \$0.31 Estimated cost File94  
 \$8.96 0.287 DialUnits File398  
 \$8.96 Estimated cost File398  
 \$1.17 0.056 DialUnits File357  
 \$1.17 Estimated cost File357  
 \$2.61 0.246 DialUnits File73  
 \$2.61 Estimated cost File73  
 \$0.57 0.126 DialUnits File50  
 \$0.57 Estimated cost File50  
 OneSearch, 18 files, 4.673 DialUnits FileOS  
 \$1.06 TELNET  
 \$52.41 Estimated cost this search  
 \$61.82 Estimated total session cost 8.223 DialUnits

### Status: Signed Off. (7 minutes)

### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)  
 Trying 31060000009998...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHHH SSSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 04.20.00D

Reconnected in file OS 26feb05 10:12:47

\* \* \*

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1951-2005/Feb W3

(c) format only 2005 The Dialog Corp.

\*File 155: Medline has been reloaded; accession numbers have changed.

Please see HELP NEWS 154.

File 654:US Pat.Full. 1976-2005/Feb 24

(c) Format only 2005 The Dialog Corp.

File 399:CA SEARCH(R) 1967-2005/UD=14209

(c) 2005 American Chemical Society

\*File 399: Use is subject to the terms of your user/customer agreement.

Alert feature enhanced for multiple files, etc. See HELP ALERT.

File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210

(c) 2005 WIPO/Univentio





Set Items Description  
S1 373 ALPHA (2N) THIONIN?  
S2 313 RD (unique items)  
S3 279736 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'  
S4 53755 'EDTA'  
S5 44607 R1-R4  
S6 79 S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)  
S7 51 S6/2002:2005  
S8 28 S6 NOT S7  
?t s8/3,kwic/19 22 27 28 3 6 7 8 10 13 17  
>>>KWIC option is not available in file(s): 398, 399

8/3,KWIC/19 (Item 19 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

4003403  
Derwent Accession: 1995-090847

#### Utility

C/ Antimicrobial proteins from Allium

; FUNGICIDES; BACTERICIDES

Inventor: Broekaert, Willem Frans, Dilbeek, BE  
Cammue, Bruno Philippe Angelo, Alsemberg, BE  
Rees, Sarah Bronwen, Bracknell, GB England

Assignee: Zeneca Limited(03), GB, England  
Zeneca Ltd GB (Code: 32757)

Examiner: LeGuyader, John L. (Art Unit: 189)

Assistant Examiner: Larson, Thomas G.

Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro

|             | Publication<br>Number | Kind | Date          | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|---------------|-----------------------|----------------|
| Main Patent | US 5773694            | A    | 19980630      | US 96591498           | 19960125       |
| PCT         | WO 9504754            |      | 19950216      | WO 94GB1636           | 19940729       |
|             |                       |      | 371:19960125  |                       |                |
|             |                       |      | 102e:19960125 |                       |                |
| Priority    |                       |      |               | GB 9316158            | 19930804       |
|             |                       |      |               | GB 9317816            | 19930827       |

Fulltext Word Count: 7927

#### Summary of the Invention:

...Rhizoctonia solani (Logemann et al, 1992, Biotechnol, 10:305-308);  
transgenic tobacco expressing a barley [ alpha ]- thionin has increased  
resistance to Pseudomonas bacterial pathogens (Carmona et al, 1993, Plant  
J, 3(3...

#### Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM EDTA and 2 mM  
thiourea. After extraction, the slurry was mixed in a WARING blender and  
...sample buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, mM  
EDTA , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v)  
dithioerythritol (DTE). Proteins were...filtration over a  
Chromaspin+TE-100 (Clontech) column equilibrated in 10 mM Tris, 1 mM  
EDTA , 300 mM NaCl, 0.05% (w/v) SDS (pH 8). RNA was subsequently removed  
by...

8/3,KWIC/22 (Item 22 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

3644278  
Derwent Accession: 1992-415779

#### Utility

C/ Antipathogenic peptides and compositions containing the same  
; HERBICIDES

Inventor: Garcia-Olmedo, Francisco, Madrid, ES  
Fernandez, Antonio M., Madrid, ES  
Assignee: Universidad Politecnica de Madrid(03), Madrid, ES  
Madrid, Universidad Politecnica de ES (Code: 36907)  
Examiner: Fox, David T. (Art Unit: 183)  
Assistant Examiner: McElwain, Elizabeth F.  
Law Firm: Wenderoth, Lind & Ponack

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 5446127            | A    | 19950829 | US 94326352           | 19941020       |
| Continuation | Abandoned             |      |          | US 93965284           | 19930125       |
| Priority     |                       |      |          | ES 911258             | 19910524       |

Fulltext Word Count: 14350

Summary of the Invention:

...such as dithiothreitol to minimize oxidation of cystein residues, and a metal chelater such as **EDTA** to prevent exposure of the protein to heavy metal ions that might inactivate the protein...suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, **ethylenediamine** propylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain...

Description of the Invention:

...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl, 10 mM **EDTA**, pH 7.5...For control purposes two standards are used in the above test that is [ **alpha** ]1/[**beta**] **thionin** from wheat endosperm and LT26-thionin from barley leaves...medium is maintained on a callus growth medium comprised of MS major, minor salts and Fe- **EDTA** (Gibco #500-1117; 4.3 g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/3,KWIC/27 (Item 2 from file: 348)

DIALOG(R) File 348:EUROPEAN PATENTS

(c) 2005 European Patent Office. All rts. reserv.

00952289

Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods

Verwendung von Thiol-Redox-Proteinen zur Reduktion des intramolekularen Disulfid-Bindung bei Proteinen, zur Besserung der Qualitat der Getreidenprodukte, des Te

Utilisation des proteines redox des groupes thiol pour la reduction des liaisonsdisulfide intramoleculaires, pour augmenter la qualite des produits cerealiers,

PATENT ASSIGNEE:

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, (221079), Office of Technology Licensing, Suite 510, 2150 Shattuck Avenue, Berkeley, California 94720-1620, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;SE)

INVENTOR:

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Lozano, Rosa, 1155 Brighton Avenue, 16, Albany, California 94706, (US)  
Jiao, Jin-an, 10555 West Flagler Street, Miami, Florida 33175, (US)  
Shin, Sungho c/o Paek Kee Yoeup,, Department of Horticulture Chungbuk National Univ., Cheongju, 360-763, (KR)

LEGAL REPRESENTATIVE:

Phelip, Bruno et al (17811), c/o Cabinet Harle & Phelip 7, rue de Madrid,  
75008 Paris, (FR)

PATENT (CC, No, Kind, Date): EP 863154 A1 980909 (Basic)

APPLICATION (CC, No, Date): EP 98201252 921008;

PRIORITY (CC, No, Date): US 776102 911012

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC;  
NL; SE

RELATED PARENT NUMBER(S) - PN (AN):

EP 672127 (EP 929218022)

INTERNATIONAL PATENT CLASS: C07K-014/415; C07K-014/76; A21D-002/26;

ABSTRACT WORD COUNT: 146

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|----------------|----------|--------|------------|
|----------------|----------|--------|------------|

|          |           |      |     |
|----------|-----------|------|-----|
| CLAIMS A | (English) | 9837 | 371 |
|----------|-----------|------|-----|

|        |           |      |       |
|--------|-----------|------|-------|
| SPEC A | (English) | 9837 | 25170 |
|--------|-----------|------|-------|

|                               |       |
|-------------------------------|-------|
| Total word count - document A | 25541 |
|-------------------------------|-------|

|                               |   |
|-------------------------------|---|
| Total word count - document B | 0 |
|-------------------------------|---|

|                                    |       |
|------------------------------------|-------|
| Total word count - documents A + B | 25541 |
|------------------------------------|-------|

...SPECIFICATION measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin ( alpha )** and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins

**Purothionin ( alpha )** from bread wheat and **purothionins ( alpha )-1** and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin ( alpha )** sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin ( alpha )-1** and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method Steps...

...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin ( alpha )**, 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin -( alpha )**, failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124...

...240). However, in contrast to purothionin-a from bread wheat, two purothionins previously not examined ( **purothionins ( alpha )-1** and (beta) from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the ( **alpha** )-type) by the SDS-PAGE fluorescence procedure (Fig. 7).

EXAMPLE 6

Quantitation of Reduction

The...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two ( **alpha** )-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate

buffer, pH 7.9 containing 10 mM **EDTA** at 30(degree)C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA** ) chromatography. Pullulanase inhibitor protein was purified as described below.

#### CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** .  
Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

**8/3,KWIC/28 (Item 3 from file: 348)**

DIALOG(R)File 348:EUROPEAN PATENTS

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00709018

#### USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS

#### VERWENDUNG VON THIOLGRUPPEN ENTHALTENDEN REDOX-PROTEINEN ZUR REDUKTION VON DISULFIDBINDUNGEN

#### UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES

#### PATENT ASSIGNEE:

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, (221072), 300 Lakeside Drive, 22nd Floor, Oakland, California 94612-3550, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;SE)

#### INVENTOR:

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KOBREHEL, Karoly Laboratoire de Technologie des Cereales (INRA) 2, place Viala, F-34060 Montpellier Cedex 01, (FR)  
YEE, Boihon, C., 3215 Primrose Lane, Walnut Creek, CA 94598, (US)  
WONG, Joshua, H., 9 Viewmont Terrace, South San Francisco, CA 94080, (US)  
LOZANO, Rosa, 1155 Brighton Avenue, 16, Albany, CA 94706, (US)  
JIAO, Jin-an, 10555 West Flagler Street, Miami, FL 33175, (US)  
SHIN, Sungho Paek Kee Yoeup Department, of Horticulture Chungbuk National University, Cheongju 360-763, (KR)

#### LEGAL REPRESENTATIVE:

Phelip, Bruno et al (17811), c/o Cabinet Harle & Phelip 7, rue de Madrid, 75008 Paris, (FR)

PATENT (CC, No, Kind, Date): EP 672127 A1 950920 (Basic)  
EP 672127 A1 960327  
EP 672127 B1 990107  
WO 9308274 930429

APPLICATION (CC, No, Date): EP 92921802 921008; WO 92US8595 921008

PRIORITY (CC, No, Date): US 776109 911012; US 935002 920825

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; SE

INTERNATIONAL PATENT CLASS: A21D-010/00; A21D-013/00; A23L-001/18;

A23L-001/168; A23L-001/172; A21D-002/26;

#### NOTE:

No A-document published by EPO

LANGUAGE (Publication,Procedural,Application): English; English; English

#### FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS B                           | (English) | 9901   | 992        |
| CLAIMS B                           | (German)  | 9901   | 1020       |
| CLAIMS B                           | (French)  | 9901   | 1208       |
| SPEC B                             | (English) | 9901   | 24864      |
| Total word count - document A      |           |        | 0          |
| Total word count - document B      |           |        | 28084      |
| Total word count - documents A + B |           |        | 28084      |

...SPECIFICATION measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known... the E. coli NADP/Thioredoxin System.

Fig. 5 is a graph showing the effect of **purothionin (alpha)** and CM-1 (alpha)-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

#### Other proteins

**Purothionin (alpha)** from bread wheat and **purothionins (alpha)-1** and (beta) from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin (alpha)** sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin (alpha)-1** and (beta) samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

#### Routine Method Steps...

...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...5, conditions were as in Fig. 4 except that the DSG proteins were omitted and **purothionin (alpha)**, 20 (mu)g or CM-1, 20 (mu)g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin -(alpha)**, failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin -(alpha)** from bread wheat, two purothionins previously not examined (**purothionins (alpha)-1** and (beta) from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the (**alpha**)-type) by the SDS-PAGE fluorescence procedure (Fig. 7).

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The...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two (**alpha**)-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30(degree)C for 2 hours. ... mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

##### CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**.

Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/3 (Item 3 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4461438 \*\*IMAGE Available

Derwent Accession: 1996-230600

#### Utility

C/ Neutralization of food allergens by thioredoxin

; CONTACTING PROTEIN WITH AMOUNT OF THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLÉOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE AND NADPH OR AMOUNT OF

**THIOREDOXIN AND DITHIOTHREITOL EFFECTIVE FOR DECREASING ALLERGENICITY OF PROTEIN; ADMINISTERING**

Inventor: Buchanan, Bob B., Berkeley, CA  
Kobrehel, Karoly, Montpellier, FR  
Yee, Boihon C., Walnut Creek, CA  
Lozano, Rosa, Madrid, ES  
Frick, Oscar L., San Francisco, CA  
Ermel, Richard W., Winters, CA

Assignee: The Regents of the University of California(02), Berkeley, CA  
California, University of Regents (Code: 13234)

Examiner: Hendricks, Keith (Art Unit: 171)

Law Firm: Flehr Hohbach Test Albritton & Herbert LLP

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6190723            | A    | 20010220 | US 9846780            | 19980323       |
| Division    | US 5792506            | A    |          | US 94326976           | 19941021       |
| CIP         | Pending               |      |          | US 94211673           | 19940412       |
| CIP         | Abandoned             |      |          | US 92935002           | 19920825       |
| CIP         | Abandoned             |      |          | US 91776109           | 19911012       |

Fulltext Word Count: 29804

**Summary of the Invention:**

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

**Description of the Invention:**

... **Purothionin [ alpha ]** from bread wheat and **purothionins [ alpha ]-1** and **[beta]** from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin [ alpha ]** sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin [ alpha ]-1** and **[beta]** samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin [ alpha ]**, 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin -[ alpha ]**, failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin -[ alpha ]** from bread wheat, two purothionins previously not examined ( **purothionins [ alpha ]-1** and **[beta]** from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the **[ alpha ]**-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two **[ alpha ]**-amylase inhibitors (DSG-1, CM-1), a cysteine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/6 (Item 6 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4413998

Derwent Accession: 1992-415779

#### Utility

C/ **Antipathogenic peptides and compositions containing same  
; NUCLEOTIDE SEQUENCES CODING AN AMINO ACID SEQUENCE ASSOCIATED WITH THE  
PREVENTION OF INFECTION BY FUNGAL AND BACTERIAL PLANT PARASITES**

Inventor: Garcia-Olmedo, Francisco, Madrid, ES

Fernandez, Antonio Molina, Madrid, ES

Assignee: Universidad Politecnica de Madrid(03), Madrid, ES

Madrid, Universidad Politecnica de ES (Code: 36907)

Examiner: McElwain, Elizabeth F. (Art Unit: 168)

Law Firm: Wenderoth, Lind & Ponack, LLP.

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 6147281            | A    | 20001114 | US 95404607           | 19950315       |
| Division     | US 5446127            | A    |          | US 94326352           | 19941020       |
| Continuation | Abandoned             |      |          | US 93965284           | 19930125       |
| Continuation | Pending               |      |          | WO 92EP1130           | 19920521       |
| Priority     |                       |      |          | ES 911258             | 19910524       |

Fulltext Word Count: 14201

#### Description of the Invention:

...such as dithiothreitol to minimize oxidation of cystein residues,  
and a metal chelater such as **EDTA** to prevent exposure of the protein to  
heavy metal ions that might inactivate the protein...suitable non-ionic  
surfactants are the water-soluble adducts of polyethylene oxide with  
polypropylene glycol, **ethylenediamine** propylene glycol and  
alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl  
chain...volumes of a buffer solution (80 ml) comprising 0.1 M Tris-HCl,  
10 mM **EDTA** , pH 7.5...For control purposes two standards are used in the  
above test that is [ **alpha** ]1/[**beta**] **thionin** from wheat endosperm and  
LT26-thionin from barley leaves...is maintained on a callus growth medium  
comprised of MS major, minor salts and Fe- **EDTA** (Gibco # 500-1117; 4.3  
g/l), MS vitamins, 100 mg/l myo-inositol, 20...

8/3,KWIC/7 (Item 7 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4377970

Derwent Accession: 1993-152468

#### Utility

C/ **Use of thiol redox proteins for reducing protein intramolecular  
disulfide bonds, for improving the quality of cereal products, dough and  
baked goods and for inactivating snake, bee and scorpion toxins**

Inventor: Buchanan, Bob B., Berkeley, CA

Kobrehel, Karoly, Montpellier, FR

Yee, Boihon C., Walnut Creek, CA

Wong, Joshua H., South San Francisco, CA

Lozano, Rosa, Madrid, ES

Jiao, Jin-an, Miami, FL

Shin, Sungho, Taejon, KR South Korea

Assignee: The Regents of the University of California(02), Oakland, CA

California, University of Regents (Code: 13234)

Examiner: Sisson, Bradley (Art Unit: 163)

Assistant Examiner: Bugaisky, Gabriele E.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6114504            | A    | 20000905 | US 95483930           | 19950607       |
| Division    | Pending               |      |          | US 211673             |                |

Fulltext Word Count: 26012

Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Drawings:

...FIG. 5 is a graph showing the effect of **purothionin** [ **alpha** ] and CM-1 [ **alpha** ]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

Description of the Invention:

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8/3,KWIC/8 (Item 8 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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4377417

Derwent Accession: 1993-152468

Utility

REASSIGNED

C/ Use of thiol redox proteins for reducing protein intramolecular disulfide bonds, for improving the quality of cereal products, dough and



**baked goods and for inactivating snake, bee and scorpion toxins**

Inventor: Buchanan, Bob B., Berkeley, CA

Kobrehel, Karoly, Montpellier, FR

Yee, Boihon C., Walnut Creek, CA

Wong, Joshua H., South San Francisco, CA

Lozano, Rosa, Madrid, ES

Jiao, Jin-an, Miami, FL

Shin, Sungho, Taejon, KR South Korea

Assignee: The Regents of the University of California(02), CA

California, University of Regents (Code: 13234)

Examiner: Sisson, Bradley (Art Unit: 163)

Assistant Examiner: Bugaisky, Gabriele E.

Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

|             | Publication<br>Number | Kind | Date          | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|---------------|-----------------------|----------------|
| Main Patent | US 6113951            | A    | 20000905      | US 94211673           | 19941121       |
| CIP         | Abandoned             |      |               | US 92935002           | 19920825       |
| CIP         | Abandoned             |      |               | US 91776109           | 19911012       |
| PCT         | WO 9308274            |      | 19930429      | WO 92US8595           | 19921008       |
|             |                       |      | 371:19941121  |                       |                |
|             |                       |      | 102e:19941121 |                       |                |

Fulltext Word Count: 28198

**Summary of the Invention:**

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area,

**Description of the Drawings:**

...FIG. 5 is a graph showing the effect of **purothionin** [ **alpha** ] and CM-1 [alpha]-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the E...

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**EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/10 (Item 10 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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4314235

Derwent Accession: 1998-216927

#### Utility

#### CERTIFICATE OF CORRECTION

C/ Manipulating nitrosative stress to kill pathologic microbes, pathologic helminths and pathologically proliferating cells or to upregulate nitrosative stress defenses

Inventor: Stamler, Jonathan S., Chapel Hill, NC

Griffith, Owen W., Milwaukee, WI

Assignee: Duke University(02), Durham, NC

The Medical College of Wisconsin Research Foundation, Inc.(02),

Milwaukee, WI

Duke University

Medical College of Wisconsin The (Code: 05187 25202)

Examiner: Weddington, Kevin E. (Art Unit: 164)

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6057367            | A    | 20000502 | US 97852490           | 19970507       |
| Provisional |                       |      |          | US 60-25819           | 19960830       |

Fulltext Word Count: 30970

#### Summary of the Invention:

...alkyl contains 1 to 10 carbon atoms. Some examples of species of this genus are [ **alpha** ]-ethyl- **buthionine** sulfoximine, [ **alpha** ]-propyl- **buthionine** sulfoximine, [ **alpha** ]-isopropyl- **buthionine** sulfoximine, and [ **alpha** ]-tert butyl- **buthionine** sulfoximine

#### Description of the Invention:

...A preferred selective inhibitor of glutathione synthesis within the above-described genus is [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine, which can be readily prepared by the method described above, i.e., by...

...and sodium cyanide to form the corresponding hydantoin, hydrolyzing the hydantoin in alkali to form [ **alpha** ]-ethyl-DL- **buthionine** , converting that compound to the corresponding sulfoximine by reaction with sodium azide in sulfuric acid. Other sulfoximines embraced by the genus described above, include, for example, [ **alpha** ]-propyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-isopropyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-tert butyl-DL- **buthionine** -SR-sulfoximine, [ **alpha** ]-ethyl-S-butyl-[delta]-thionorvaline sulfoximine, and [ **alpha** ]-propyl-S-butyl-[delta]-thionorvaline sulfoximine. Also...where the [ **alpha** ]-alkyl is ethyl and/or the S-alkyl is butyl, e.g., [ **alpha** ]-ethyl-L- **buthionine** -S-sulfoximine, with the dosages and routes of administration described above for these applying here...mM MgCl<sub>2</sub>, 7.5 mM ATP, 7.5 mM phosphoenolpyruvate, 0.3 mM **EDTA** , 10 mM L-glutamate, 10 mM L-[ **alpha** ]-aminobutyrate (an L-cysteine analog), 0.3...2, 142 mM KCl, 36 mM MgCl<sub>2</sub>, 10 mM ATP, 0.4 mM **EDTA** , various amounts of sulfoximine, and gamma-GCS. At intervals, 50 [mu]l aliquots were removed similar level of inactivation was achieved with 2 mM with [ **alpha** ]-ethyl-DL- **buthionine** -SR-sulfoximine

(DL-SR-[alpha]-ethyl-BSO), synthesized by the method described in Griffith, O...

...but only the L-S isomer is active as an enzyme inhibitor. The concentration of [ alpha ]-ethyl-L- buthionine -S-sulfoximine is [difference]1/4 of the total concentration or [difference]500 [mu]M...

...large to bind to that enzyme (see said FIG. 5 mentioned above). In separate studies, [ alpha ]-ethyl-DL- buthionine -SR-sulfoximine was compared to L-buthionine-S-sulfoximine as an inhibitor of mammalian gamma ...

...protocol similar to that described here for the E. coli enzyme, it was found that [ alpha ]-ethyl-DL- buthionine -SR-sulfoximine was 0.025% as effective as L-buthionine-S-sulfoximine as an inhibitor of the mammalian enzyme. Expressed on the basis of the active isomer, [ alpha ]-ethyl-L- buthionine -s-sulfoximine, [ alpha ]-ethyl-BSO has about A patient with E. coli caused gastroenteritis with bloody diarrhea is administered [ alpha ]-ethyl-L- buthionine -S-sulfoximine (hereinafter [alpha]-ethyl BSO) orally at a dose of 10 mmol/kg body...

...day. Bloody diarrhea resolved over four days. Similar results obtained using the same dose of [ alpha ]-ethyl-L- buthionine -S-sulfoximine given intravenously at the same dose...

Non-exemplary or Dependent Claim(s):

...7. The method of claim 6 wherein the manipulator of nitrosative stress is [ alpha ]-ethyl-L- buthionine -S-sulfoximine...

8/3,KWIC/13 (Item 13 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4198164

Derwent Accession: 1999-288106

#### Utility

C/ Increasing the digestibility of food proteins by thioredoxin reduction ; FOOD TREATMENT WITH THIOREDOXIN, NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-THIOREDOXIN REDUCTASE, REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH)AND ADMINISTERING TREATED FOOD TO ANIMAL

Inventor: Buchanan, Bob B., Berkeley, CA

del Val, Gregorio, Saint-Aubin/NE, CH

Lozano, Rosa M., Madrid, ES

Jiao, Jin-an, Ft. Lauderdale, FL

Wong, Joshua H., South San Francisco, CA

Yee, Boihon C., Walnut Creek, CA

Assignee: The Regents of the University of California(02), Oakland, CA

California, University of Regents (Code: 13234)

Examiner: Hendricks, Keith D. (Art Unit: 171)

Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 5952034            | A    | 19990914 | US 97953703           | 19971017       |
| CIP         | US 5792506            | A    |          | US 94326976           | 19941021       |
| CIP         | Pending               |      |          | US 94211673           | 19940412       |
| CIP         | Abandoned             |      |          | US 92935002           | 19920825       |
| CIP         | Abandoned             |      |          | US 91776109           | 19911012       |

Fulltext Word Count: 35406

#### Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory

failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

Description of the Invention:

... **Purothionin** [ **alpha** ] from bread wheat and **purothionins** [ **alpha** ]-1 and [ **beta** ] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [ **beta** ] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume ... same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [ **beta** ] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...with 30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

8/3,KWIC/17 (Item 17 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

4022853  
Derwent Accession: 1996-230600

Utility

REASSIGNED

C/ Neutralization of food allergens by thioredoxin  
; DISULFIDE BONDS ARE REDUCED TO SULFHYDRYL DECREASING ALLERGENICITY OF FEEDS

Inventor: Buchanan, Bob B., Berkeley, CA  
Kobrehel, Karoly, Montpellier, FR  
Yee, Boihon C., Walnut Creek, CA  
Lozano, Rosa, Madrid, ES  
Frick, Oscar L., San Francisco, CA  
Ermel, Richard W., Winters, CA

Assignee: The Regents of the University of California(02), Oakland, CA  
California, University of Regents (Code: 13234)

Examiner: Grimes, Eric (Art Unit: 184)

Combined Principal Attorneys: Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP

| Publication<br>Number | Kind | Date  | Application<br>Number | Filing<br>Date |
|-----------------------|------|-------|-----------------------|----------------|
| -----                 | ---  | ----- | -----                 | -----          |

|             |            |   |          |             |          |
|-------------|------------|---|----------|-------------|----------|
| Main Patent | US 5792506 | A | 19980811 | US 94326976 | 19941021 |
| CIP         | Pending    |   |          | US 94211673 | 19940412 |
| CIP         | Abandoned  |   |          | US 92935002 | 19920825 |
| CIP         | Abandoned  |   |          | US 91776109 | 19911012 |

Fulltext Word Count: 30535

#### Summary of the Invention:

...measures are taken to minimize shock, renal failure and respiratory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...

#### Description of the Invention:

...Purothionin a from bread wheat and **purothionins** [ **alpha** ]-1 and [beta] from durum wheat were kind gifts from Drs. D. D. Kasarda and B. L. Jones, respectively. The **purothionin** [ **alpha** ] sample contained two members of the purothionin family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** [ **alpha** ]-1 and [beta] samples were both homogeneous in SDS-polyacrylamide gel electrophoresis...was carried out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0.7 ...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** [ **alpha** ], 20 [mu]g or CM-1, 20 [mu]g was used). The results thus confirmed...confirmation of earlier results, thioredoxin-reduced purothionin consistently activated FBPase and the type tested earlier, **purothionin** -[ **alpha** ], failed to activate NADP-MDH (Table I) (Wada, K., et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -[ **alpha** ] from bread wheat, two purothionins previously not examined ( **purothionins** [ **alpha** ]-1 and [beta] from durum wheat) detectably activated NADP-MDH (Table I). The two durum...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the [ **alpha** ]-type) by the SDS-PAGE fluorescence procedure...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two [ **alpha** ]-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants (corn kernel...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30[degree(s)] C. for 2 hours. The concentrations of thioredoxin, NTR, and NADPH...

...mg/ml, 0.02 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below...

...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA**.  
Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...  
?e purothionin

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E1  | 1     | PUROTHIONEIN                                   |
| E2  | 1     | PUROTHIONIM                                    |
| E3  | 525   | *PURUTHIONIN                                   |
| E4  | 1     | PUROTHIONIN (RYE GENE PUR-R1 PRECURSOR REDUCED |
| E5  | 1     | PUROTHIONIN (WHEAT GENE PUR-A1 PRECURSOR REDUC |
| E6  | 1     | PUROTHIONIN (WHEAT GENE PUR-B1 PRECURSOR REDUC |
| E7  | 1     | PUROTHIONIN (WHEAT GENE PUR-D1 PRECURSOR REDUC |
| E8  | 4     | PUROTHIONIN A I                                |
| E9  | 16    | PUROTHIONIN A I (REDUCED)                      |
| E10 | 1     | PUROTHIONIN A II                               |
| E11 | 1     | PUROTHIONIN A II (REDUCED)                     |
| E12 | 1     | PUROTHIONIN CONTENT                            |

Enter P or PAGE for more

?p

| Ref | Items | Index-term                                     |
|-----|-------|------------------------------------------------|
| E13 | 2     | PUROTHIONIN DERIVATIVE                         |
| E14 | 1     | PUROTHIONIN HOMOLOG II (BARLEY ENDOSPERM REDUC |
| E15 | 1     | PUROTHIONIN PROTEIN-PROTEIN INTERACTION        |
| E16 | 2     | PUROTHIONIN-ALPHA                              |
| E17 | 1     | PUROTHIONIN-BETA                               |
| E18 | 1     | PUROTHIONIN, .ALPHA.-                          |
| E19 | 1     | PUROTHIONIN, .BETA.-                           |
| E20 | 1     | PUROTHIONIN, HYDROCHLORIDE                     |
| E21 | 24    | PUROTHIONINE                                   |
| E22 | 1     | PUROTHIONINELUTED                              |
| E23 | 5     | PUROTHIONINES                                  |
| E24 | 1     | PUROTHIONINLIKE                                |

Enter P or PAGE for more

?p

| Ref | Items | Index-term                          |
|-----|-------|-------------------------------------|
| E25 | 1     | PUROTHIONINOX                       |
| E26 | 1     | PUROTHIONINRD                       |
| E27 | 295   | PUROTHIONINS                        |
| E28 | 5     | PUROTHIONINS, .ALPHA.-              |
| E29 | 10    | PUROTHIONINS, .ALPHA.-HORDOTHIONINS |
| E30 | 11    | PUROTHIONINS, .ALPHA.1-             |
| E31 | 3     | PUROTHIONINS, .ALPHA.2-             |
| E32 | 16    | PUROTHIONINS, .BETA.-               |
| E33 | 3     | PUROTHIONINS, .BETA.-HORDOTHIONINS  |
| E34 | 3     | PUROTHIONINS, .GAMMA.1-             |
| E35 | 1     | PUROTHIONINS, .GAMMA.2-             |
| E36 | 3     | PUROTHIONINS, AVENOTHIONINS         |

Enter P or PAGE for more

?s e3-e33

|     |                                                |
|-----|------------------------------------------------|
| 525 | PUROTHIONIN                                    |
| 1   | PUROTHIONIN (RYE GENE PUR-R1 PRECURSOR REDUCED |
| 1   | PUROTHIONIN (WHEAT GENE PUR-A1 PRECURSOR REDUC |
| 1   | PUROTHIONIN (WHEAT GENE PUR-B1 PRECURSOR REDUC |
| 1   | PUROTHIONIN (WHEAT GENE PUR-D1 PRECURSOR REDUC |
| 4   | PUROTHIONIN A I                                |
| 16  | PUROTHIONIN A I (REDUCED)                      |
| 1   | PUROTHIONIN A II                               |
| 1   | PUROTHIONIN A II (REDUCED)                     |
| 1   | PUROTHIONIN CONTENT                            |
| 2   | PUROTHIONIN DERIVATIVE                         |
| 1   | PUROTHIONIN HOMOLOG II (BARLEY ENDOSPERM REDUC |
| 1   | PUROTHIONIN PROTEIN-PROTEIN INTERACTION        |
| 2   | PUROTHIONIN-ALPHA                              |
| 1   | PUROTHIONIN-BETA                               |
| 1   | PUROTHIONIN, .ALPHA.-                          |
| 1   | PUROTHIONIN, .BETA.-                           |
| 1   | PUROTHIONIN, HYDROCHLORIDE                     |
| 24  | PUROTHIONINE                                   |
| 1   | PUROTHIONINELUTED                              |
| 5   | PUROTHIONINES                                  |
| 1   | PUROTHIONINLIKE                                |
| 1   | PUROTHIONINOX                                  |
| 1   | PUROTHIONINRD                                  |
| 295 | PUROTHIONINS                                   |
| 5   | PUROTHIONINS, .ALPHA.-                         |
| 10  | PUROTHIONINS, .ALPHA.-HORDOTHIONINS            |
| 11  | PUROTHIONINS, .ALPHA.1-                        |
| 3   | PUROTHIONINS, .ALPHA.2-                        |
| 16  | PUROTHIONINS, .BETA.-                          |
| 3   | PUROTHIONINS, .BETA.-HORDOTHIONINS             |

S9

689 E3-E33

?p

| Ref | Items | Index-term                  |
|-----|-------|-----------------------------|
| E37 | 1     | PUROTHIONINS,HOMOLOGS       |
| E38 | 14    | PUROTHIONINS,HORDOTHIONINS  |
| E39 | 1     | PUROTHIONINS,SECALETHIONINS |
| E40 | 1     | PUROTHIONIRL                |
| E41 | 1     | PUROTHIONNIN                |
| E42 | 1     | PUROTHIORINS                |
| E43 | 2     | PUROTI                      |
| E44 | 1     | PUROTID                     |
| E45 | 15    | PUROTIE                     |
| E46 | 1     | PUROTIONIN                  |
| E47 | 1     | PUROTNYCIN                  |
| E48 | 1     | PUROTOC                     |

Enter P or PAGE for more

?s e42

S10 1 'PUROTHIORINS'

?ds

| Set | Items  | Description                                               |
|-----|--------|-----------------------------------------------------------|
| S1  | 373    | ALPHA (2N) THIONIN?                                       |
| S2  | 313    | RD (unique items)                                         |
| S3  | 279736 | 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION' |
| S4  | 53755  | 'EDTA'                                                    |
| S5  | 44607  | R1-R4                                                     |
| S6  | 79     | S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)      |
| S7  | 51     | S6/2002:2005                                              |
| S8  | 28     | S6 NOT S7                                                 |
| S9  | 689    | E3-E33                                                    |
| S10 | 1      | 'PUROTHIORINS'                                            |

?s s9 or s10

689 S9

1 S10

S11 689 S9 OR S10

?s s11 and (edta? or ethylene? or ethylenediamine? or edta)

>>>File 654 processing for ETHYLENE? stopped at ETHYLENEDIAMINOTETRAACETATE

>>>File 399 processing for ETHYLENE? stopped at ETHYLENEDIAMINIUM

>>>File 349 processing for ETHYLENE? stopped at ETHYLENEFLUOROPOLYMER

>>>File 340 processing for ETHYLENE? stopped at ETHYLENESULFINYL

>>>File 348 processing for ETHYLENE? stopped at ETHYLENELAURYLAMINEETHER

689 S11

280289 EDTA?

1981588 ETHYLENE?

198685 ETHYLENEDIAMINE?

279736 EDTA

S12 128 S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)

?s s12/2002:2005

>>>One or more prefixes are unsupported

>>> or undefined in one or more files.

>>>Year ranges not supported in one or more files

128 S12

15341873 PY=2002 : PY=2005

S13 50 S12/2002:2005

?s s12 not s13

128 S12

50 S13

S14 78 S12 NOT S13

?ds

| Set | Items  | Description                                               |
|-----|--------|-----------------------------------------------------------|
| S1  | 373    | ALPHA (2N) THIONIN?                                       |
| S2  | 313    | RD (unique items)                                         |
| S3  | 279736 | 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION' |
| S4  | 53755  | 'EDTA'                                                    |
| S5  | 44607  | R1-R4                                                     |

S6 79 S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)  
 S7 51 S6/2002:2005  
 S8 28 S6 NOT S7  
 S9 689 E3-E33  
 S10 1 'PUROTHIORINS'  
 S11 689 S9 OR S10  
 S12 128 S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)  
 S13 50 S12/2002:2005  
 S14 78 S12 NOT S13  
 ?s s14 not s8  
     78 S14  
     28 S8  
     S15 70 S14 NOT S8  
 ?t s15/6/all

15/6/1 (Item 1 from file: 155)  
 08856644 PMID: 2610351  
**Histochemical localization of cysteine-rich proteins by tissue printing on nitrocellulose.**  
 Nov 1 1989

15/6/2 (Item 1 from file: 654)  
 0004925643  
 Derwent Accession: 2002-010726  
**Compositions and methods for identifying and targeting cancer cells of alimentary canal origin**  
 Fulltext Word Count: 30220  
 Number of Claims: 38  
 Exemplary or Independent Claim Number(s): 1,11,21,25,29,33,34,37

15/6/3 (Item 2 from file: 654)  
 0004918035  
 Derwent Accession: 2002-381264  
**Compositions and methods for identifying and targeting cancer cells of alimentary canal origin**  
 Fulltext Word Count: 30022  
 Number of Claims: 38  
 Exemplary or Independent Claim Number(s): 1,11,21,25,29,33,34,37

15/6/4 (Item 3 from file: 654)  
 0004903757 \*\*IMAGE Available  
 Derwent Accession: 1993-100978  
**Biocidal proteins**  
 Fulltext Word Count: 14428  
 Number of Claims: 43  
 Exemplary or Independent Claim Number(s):  
 1,6,7,8,9,10,11,12,13,14,15,16,17,18,20,25,36,37  
 Number of Drawing Sheets: 42  
 Number of Figures: 42

15/6/5 (Item 4 from file: 654)  
 4547046  
 Derwent Accession: 1995-178646  
**Utility**  
**C/ Imaging of colorectal cancer using ST receptor binding compounds ; VISUALIZING TUMOR CELLS; ADMINISTER RADIOACTIVE PARTICLES TO HUMANS AND DETECT POSITIONING AND ACCUMULATION OF RADIOACTIVE PARTICLES IN BODY**  
 Fulltext Word Count: 28331  
 Number of Claims: 17



Exemplary or Independent Claim Number(s): 11  
Number of US cited patent references: 10  
Number of non-US cited patent references: 1  
Number of non-patent cited references: 70

15/6/6 (Item 5 from file: 654)

4458415 \*\*IMAGE Available

Derwent Accession: 1993-100978

Utility

C/ Biocidal proteins

; AMINO ACID SEQUENCES OF MICROBIOCIDAL PROTEINS; FOR TRANSGENIC PLANTS  
WITH INCREASED DISEASE RESISTANCE

Fulltext Word Count: 11346

Number of Claims: 2

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 39

Number of Figures: 60

Number of US cited patent references: 4

Number of non-US cited patent references: 2

Number of non-patent cited references: 15

15/6/7 (Item 6 from file: 654)

4458048

Derwent Accession: 1998-456873

Utility

C/ Methods of identifying and detecting pancreatic cancer

; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN  
TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS,  
PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS  
A TUMOR

Fulltext Word Count: 23168

Number of Claims: 27

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 55

Number of non-patent cited references: 35

15/6/8 (Item 7 from file: 654)

4427576

Derwent Accession: 2000-687452

Utility

C/ X-ray guided drug delivery

; TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION

Fulltext Word Count: 26146

Number of Claims: 104

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 9

Number of non-US cited patent references: 1

Number of non-patent cited references: 4

15/6/9 (Item 8 from file: 654)

4384858

Derwent Accession: 1999-180474

Utility

C/ Compositions that specifically bind to colorectal cancer cells and  
methods of using the same

; IN VITRO METHOD OF DIAGNOSING METASTASIZED COLORECTAL CANCER BY DETECTING  
GENE EXPRESSION OF COLORECTAL CANCER-ASSOCIATED TRANSCRIPT-1, AN ALTERNATE  
FORM OF HEAT-STABLE TOXIN RECEPTOR, IN CELLS OF SAMPLE

Fulltext Word Count: 34307

Number of Claims: 11  
Exemplary or Independent Claim Number(s): 7  
Number of Drawing Sheets: 1  
Number of Figures: 1  
Number of US cited patent references: 49  
Number of non-patent cited references: 64

15/6/10 (Item 9 from file: 654)

4317418

Derwent Accession: 1995-178646

**Utility**

C/ Compositions that specifically bind to colorectal cancer cells and methods of using the same  
; CONJUGATED COMPOUNDS WHICH COMPRISE AN ST RECEPTOR BINDING MOIETY AND A RADIOSTABLE ACTIVE MOIETY

Fulltext Word Count: 39301

Number of Claims: 10

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 12

Number of non-US cited patent references: 1

Number of non-patent cited references: 76

15/6/11 (Item 10 from file: 654)

4239629

Derwent Accession: 1994-249225

**Utility**

C/ High lysine derivatives of [alpha]-hordothionin  
; FOR TRANSFORMED PLANTS AND CELLS WITH ENHANCED LYSINE CONTENT AND WHICH SUPPRESS AND KILL PLANT PATHOGENS INCLUDING FUNGI

Fulltext Word Count: 4619

Number of Claims: 21

Exemplary or Independent Claim Number(s): 1,4,6,7,10

Number of Drawing Sheets: 2

Number of Figures: 4

Number of US cited patent references: 1

Number of non-US cited patent references: 9

Number of non-patent cited references: 14

15/6/12 (Item 11 from file: 654)

4235015

Derwent Accession: 1994-183512

**Utility**

C/ Transgenic plants expressing biocidal proteins  
; ISOLATED ANTIMICROBIAL PROTEIN HAVING AMINO ACID SEQUENCECONTAINING COMMON CYSTEINE/GLYCINE DOMAIN OF CHITIN BINDINGPLANT PROTEINS; AGICULTURAL AND PHARMACEUTICAL APPLICATIONS AS FUNGICIDES OR BACETRICIDES

Fulltext Word Count: 13574

Number of Claims: 20

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 17

Number of Figures: 29

Number of US cited patent references: 8

Number of non-patent cited references: 7

15/6/13 (Item 12 from file: 654)

4187951

Derwent Accession: 1992-331736

**Utility**

C/ Biocidal proteins  
; TRANSFORMED BIOLOGICAL PROTEIN

Fulltext Word Count: 6005  
Number of Claims: 3  
Exemplary or Independent Claim Number(s): 1,3  
Number of Drawing Sheets: 11  
Number of Figures: 12  
Number of US cited patent references: 3  
Number of non-US cited patent references: 1  
Number of non-patent cited references: 3

**15/6/14 (Item 13 from file: 654)**

4163914

Derwent Accession: 1995-246394

**Utility**

**C/ Transformed plants expressing antimicrobial proteins  
; ANTIMICROBIAL PROTEIN CAPABLE OF ISOLATION FROM SEEDS OF HEUCHERA OR  
AESCULUS**

Fulltext Word Count: 5637

Number of Claims: 8

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 5

Number of Figures: 9

Number of non-US cited patent references: 2

Number of non-patent cited references: 7

**15/6/15 (Item 14 from file: 654)**

4118486

Derwent Accession: 1995-178646

**Utility**

**C/ Methods of treating metastatic colorectal cancer with ST receptor  
binding compounds  
; RADIOACTIVE THERAPEUTIC AGENT, RECEPTOR BINDING MOIETY**

Fulltext Word Count: 33695

Number of Claims: 58

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 27

Number of non-US cited patent references: 1

Number of non-patent cited references: 83

**15/6/16 (Item 15 from file: 654)**

4085182

Derwent Accession: 1997-480228

**Utility**

**C/ Alteration of amino acid compositions in seeds**

Fulltext Word Count: 12377

Number of Claims: 28

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 6

Number of Figures: 6

Number of US cited patent references: 1

Number of non-US cited patent references: 3

Number of non-patent cited references: 33

**15/6/17 (Item 16 from file: 654)**

4057665

Derwent Accession: 1993-100978

**Utility**

**C/ Biocidal proteins  
; ANTIFUNGAL, ISOLATED FROM PLANT SEEDS**

Fulltext Word Count: 11956  
Number of Claims: 19  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 39  
Number of Figures: 42  
Number of US cited patent references: 2  
Number of non-US cited patent references: 2  
Number of non-patent cited references: 13

**15/6/18 (Item 17 from file: 654)**

3992551

Derwent Accession: 1996-188141

**Utility**

**C/ Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocorticoid receptor type II and viral protein R interacting protein  
; DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I PROTEIN**

Fulltext Word Count: 15683  
Number of Claims: 5  
Exemplary or Independent Claim Number(s): 1  
Number of US cited patent references: 2  
Number of non-patent cited references: 46

**15/6/19 (Item 18 from file: 654)**

3979152

Derwent Accession: 1995-246394

**Utility**

**C/ Antimicrobial proteins  
; PLANT EXTRACTS**

Fulltext Word Count: 5652  
Number of Claims: 5  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 5  
Number of Figures: 5  
Number of non-US cited patent references: 2  
Number of non-patent cited references: 1

**15/6/20 (Item 19 from file: 654)**

3913150

Derwent Accession: 1994-183512

**Utility**

**C/ DNA encoding biocidal proteins  
; DNA SEQUENCES IN PROTEINS AND VECTORS IN CELLS**

Fulltext Word Count: 14086  
Number of Claims: 60  
Exemplary or Independent Claim Number(s): 1  
Number of Drawing Sheets: 17  
Number of Figures: 29  
Number of US cited patent references: 3  
Number of non-patent cited references: 6

**15/6/21 (Item 20 from file: 654)**

3910716 \*\*IMAGE Available

Derwent Accession: 1992-331736

**Utility**

**C/ Biocidal proteins  
; GENETIC ENGINEERING**

Fulltext Word Count: 6067  
Number of Claims: 8

Exemplary or Independent Claim Number(s): 1,6  
Number of Drawing Sheets: 11  
Number of Figures: 22  
Number of US cited patent references: 3  
Number of non-US cited patent references: 1  
Number of non-patent cited references: 3

**15/6/22 (Item 21 from file: 654)**

3910711

Derwent Accession: 1993-100978

**Utility**

**C/ Biocidal proteins**

**; GENETIC ENGINEERING**

Fulltext Word Count: 11738

Number of Claims: 13

Exemplary or Independent Claim Number(s): 1,9

Number of Drawing Sheets: 39

Number of Figures: 44

Number of US cited patent references: 2

Number of non-US cited patent references: 2

Number of non-patent cited references: 12

**15/6/23 (Item 22 from file: 654)**

3811293

Derwent Accession: 1994-183512

**Utility**

**C/ Biocidal proteins**

**; BACTERICIDES, FUNGICIDES**

Fulltext Word Count: 14080

Number of Claims: 25

Exemplary or Independent Claim Number(s): 3

Number of Drawing Sheets: 17

Number of Figures: 29

Number of US cited patent references: 4

Number of non-patent cited references: 6

**15/6/24 (Item 23 from file: 654)**

3746704

Derwent Accession: 1993-100978

**Utility**

**CM/ Biocidal proteins**

**; ANTIFUNGAL OR ANTIBACTERIAL AGENTS**

Fulltext Word Count: 11737

Number of Claims: 14

Exemplary or Independent Claim Number(s): 1,14

Number of Drawing Sheets: 39

Number of Figures: 42

Number of US cited patent references: 2

Number of non-US cited patent references: 2

Number of non-patent cited references: 13

**15/6/25 (Item 24 from file: 654)**

3725121

Derwent Accession: 1995-178646

**Utility**

**C/ ST receptor binding compounds and methods of using the same**

**; IMAGING METASTASIZED COLORECTAL CANCER, TOXTIN PEPTIDES LESS THAN 25 UNITS**

Fulltext Word Count: 28768

Number of Claims: 22  
Exemplary or Independent Claim Number(s): 1  
Number of US cited patent references: 1  
Number of non-patent cited references: 51

**15/6/26 (Item 25 from file: 654)**

3720578

Derwent Accession: 1994-183512

**Utility**

**C/ Biocidal proteins from plants  
; CHITINASES, FUNGICIDES**

Fulltext Word Count: 13401

Number of Claims: 12

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 17

Number of Figures: 29

Number of US cited patent references: 3

Number of non-patent cited references: 6

**15/6/27 (Item 26 from file: 654)**

3685299 \*\*IMAGE Available

Derwent Accession: 1992-331736

**Utility**

**C/ Biocidal proteins**

Fulltext Word Count: 6028

Number of Claims: 5

Exemplary or Independent Claim Number(s): 1

Number of Drawing Sheets: 11

Number of Figures: 22

Number of US cited patent references: 2

Number of non-US cited patent references: 1

Number of non-patent cited references: 3

**15/6/28 (Item 27 from file: 654)**

3181895

Derwent Accession: 1989-220454

**Utility**

**C/ Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol  
peptides in hair care preparations**

Fulltext Word Count: 3722

Number of Claims: 11

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 6

**15/6/29 (Item 28 from file: 654)**

3045751

Derwent Accession: 1987-158828

**Utility**

**C/ Thioredoxin shufflease and use thereof**

Fulltext Word Count: 6059

Number of Claims: 10

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 1

Number of non-patent cited references: 4

**15/6/30 (Item 29 from file: 654)**

3034492

Derwent Accession: 1989-220454

**Utility**

**C/ Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations  
; SYNERGISTIC MIXTURE WITH SULFITES AND BISULFITES APPLIED**

Fulltext Word Count: 3257

Number of Claims: 1

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 1

Number of non-US cited patent references: 2

Number of non-patent cited references: 1

**15/6/31 (Item 30 from file: 654)**

2902171

Derwent Accession: 1987-258442

**Utility**

**C/ Method and ophthalmic composition for the prevention and reversal of cataracts  
; TOPICAL ADMINISTRATION OF THIOREDOXIN**

Fulltext Word Count: 3845

Number of Claims: 8

Exemplary or Independent Claim Number(s): 1

Number of US cited patent references: 1

Number of non-patent cited references: 14

**15/6/32 (Item 1 from file: 349)**

00851452

**PRODUCTION AND USE OF PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENECITY  
VARIANTS DE PROTEINES A IMMUNOGENICITE MODIFIEE**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 146189

Publication Year: 2001

**15/6/33 (Item 2 from file: 349)**

00840858

**COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS  
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES  
CANCEREUSES**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 37104

Publication Year: 2001

**15/6/34 (Item 3 from file: 349)**

00840857

**COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS OF  
ALIMENTARY CANAL ORIGIN  
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES  
CANCEREUSES PROVENANT DU TUBE DIGESTIF**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 27589

Publication Year: 2001

15/6/35 (Item 4 from file: 349)

00830501

**MEMBRANE ESTROGEN RECEPTOR-DIRECTED THERAPY IN BREAST CANCER**

**THERAPIE DIRIGEE SUR LE RECEPTEUR MEMBRANAIRE DES OESTROGENES, DANS LE  
CANCER DU SEIN**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 28454

Publication Year: 2001

15/6/36 (Item 5 from file: 349)

00784061

**NUCLEIC ACID SEQUENCES ENCODING CELL WALL-DEGRADING ENZYMES AND USE TO  
ENGINEER RESISTANCE TO FUSARIUM AND OTHER PATHOGENS**

**SEQUENCES D'ACIDE NUCLEIQUE CODANT POUR DES ENZYMES DEGRADANT LES PAROIS  
CELLULAIRES ET LEUR UTILISATION POUR CREER UNE RESISTANCE AU FUSARIUM  
ET A D'AUTRES PATHOGENES**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 40894

Publication Year: 2001

15/6/37 (Item 6 from file: 349)

00754251

**X-RAY GUIDED DRUG DELIVERY**

**ADMINISTRATION DE MEDICAMENT GUIDEE PAR RAYON X**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 41390

Publication Year: 2000

15/6/38 (Item 7 from file: 349)

00746101

**BARLEY GENE FOR THIOREDOXIN AND NADP-THIOREDOXIN REDUCTASE**

**GENE D'ORGE POUR REDUCTASE DE THIOREDOXINE ET DE THIOREDOXINE NADP**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 51098

Publication Year: 2000

15/6/39 (Item 8 from file: 349)

00745163 \*\*Image available\*\*

**PLANTS TRANSFORMED WITH THIOREDOXIN**

**VALORISATION DE GRAINES ET DE SEMENCES TRANSFORMEES PAR THIOREDOXINE**

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description



Claims  
Fulltext Word Count: 34481  
Publication Year: 2000

15/6/40 (Item 9 from file: 349)  
00731623 \*\*Image available\*\*  
**ALLEVIATION OF THE ALLERGENIC POTENTIAL OF AIRBORNE AND CONTACT ALLERGENS  
BY THIOREDOXIN**  
**DIMINUTION DU POTENTIEL ALLERGENIQUE D'ALLERGENES PORTES PAR L'AIR OU  
AGISSANT PAR CONTACT A L'AIDE DE THIOREDOXINE**  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 44560  
Publication Year: 2000

15/6/41 (Item 10 from file: 349)  
00577322  
**SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED  
THEREBY**  
**FRAGMENTS D'ADN DETERMINEES SELON LEUR SEQUENCE ET POLYPEPTIDES  
CORRESPONDANTS CODES PAR LESDITS FRAGMENTS**  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 226130  
Publication Year: 2000

15/6/42 (Item 11 from file: 349)  
00517693 \*\*Image available\*\*  
**RECOMBINANT MAJOR ALLERGEN OF THE POLLEN OF ARTEMISIA VULGARIS (MUGWORT)  
ALLERGENE PRINCIPAL RECOMBINE DU POLLEN D'i (ARTEMISIA VULGARIS) (ARMOISE)**  
Publication Language: German  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 3464  
Publication Year: 1999

15/6/43 (Item 12 from file: 349)  
00508857  
**ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS  
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES**  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 7355  
Publication Year: 1999

15/6/44 (Item 13 from file: 349)  
00488770 \*\*Image available\*\*  
**INCREASING THE DIGESTIBILITY OF FOOD PROTEINS BY THIOREDOXIN REDUCTION  
AMELIORATION DE LA DIGESTIBILITE DES PROTEINES ALIMENTAIRES PAR REDUCTION  
PAR LA THIOREDOXINE**  
Publication Language: English  
Fulltext Availability:  
Detailed Description

Claims  
Fulltext Word Count: 38007  
Publication Year: 1999

15/6/45 (Item 14 from file: 349)  
00476374

**COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME**

**COMPOSITIONS QUI SE LIENT SPECIFIQUEMENT AUX CELLULES CANCEREUSES COLORECTALES ET UTILISATION DE CES COMPOSITIONS**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 36129

Publication Year: 1999

15/6/46 (Item 15 from file: 349)  
00445243

**COMPOSITIONS THAT BIND TO PANCREATIC CANCER CELLS AND METHODS OF USING THE SAME**

**COMPOSITIONS QUI SE FIXENT SUR LES CELLULES CANCEREUSES PANCREATIQUES ET LEUR MODE D'UTILISATION**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23391

Publication Year: 1998

15/6/47 (Item 16 from file: 349)  
00416399

**PEPTIDE WITH INHIBITORY ACTIVITY TOWARDS PLANT PATHOGENIC FUNGI**

**PEPTIDE POSSEDANT UNE ACTION INHIBITRICE A L'ENCONTRE DE CHAMPIGNONS PATHOGENES DE PLANTES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20033

Publication Year: 1998

15/6/48 (Item 17 from file: 349)  
00394280 \*\*Image available\*\*

**ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS**

**MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 15668

Publication Year: 1997

15/6/49 (Item 18 from file: 349)  
00359094

**PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES**

**THIONINE PYRULARIA CONTENANT DES IMMUNOTOXINES AINSI QUE DES CONJUGUES SIMILAIRES AUX IMMUNOTOXINES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 12214  
Publication Year: 1996

15/6/50 (Item 19 from file: 349)  
00330288

**NEUTRALIZATION OF FOOD ALLERGENS BY THIOREDOXIN**  
**NEUTRALISATION D'ALLERGENES ALIMENTAIRES PAR LA THIOREDOXINE**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32222

Publication Year: 1996

15/6/51 (Item 20 from file: 349)  
00326460

**COMPOSITIONS AND METHODS FOR THE ABROGATION OF CELLULAR PROLIFERATION**  
**UTILIZING THE HUMAN IMMUNODEFICIENCY VIRUS Vpr PROTEIN**

**COMPOSITIONS ET PROCEDES PERMETTANT D'INTERROMPRE UNE PROLIFERATION**  
**CELLULAIRE A L'AIDE DE LA PROTEINE Vpr DU VIH**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19863

Publication Year: 1996

15/6/52 (Item 21 from file: 349)  
00300078

**ANTIMICROBIAL PROTEINS**

**PROTEINES ANTIMICROBIENNES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 7917

Publication Year: 1995

15/6/53 (Item 22 from file: 349)  
00293545

**COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS**  
**OF USING THE SAME**

**COMPOSITIONS SE FIXANT SPECIFIQUEMENT A DES CELLULES CANCEREUSES**  
**COLO-RECTALES ET PROCEDES D'UTILISATION**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 42481

Publication Year: 1995

15/6/54 (Item 23 from file: 349)  
00286605

**ANTIMICROBIAL PROTEINS**

**PROTEINES ANTIMICROBIENNES**

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 11516

Publication Year: 1995

15/6/55 (Item 24 from file: 349)

00286604

**COMPOSITIONS OF FUSION PROTEINS CONTAINING METALLOTHIONEIN AND  
TARGETING-PROTEIN STRUCTURAL COMPONENTS**

**COMPOSITIONS DE PROTEINES DE FUSION CONTENANT DES COMPOSANTS STRUCTURAUX DE  
METALLOTHIONEINE ET DE PROTEINE DE CIBLAGE**

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 9406

Publication Year: 1995

15/6/56 (Item 25 from file: 349)

00267908 \*\*Image available\*\*

**HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN**

**DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 5778

Publication Year: 1994

15/6/57 (Item 26 from file: 349)

00234016

**USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS**

**UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES  
LIAISONS BISULFURES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 33674

Publication Year: 1993

15/6/58 (Item 27 from file: 349)

00230900

**BIOCIDAL PROTEINS**

**PROTEINES BIOCIDES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 13654

Publication Year: 1993

15/6/59 (Item 28 from file: 349)

00230335

**BIOCIDAL PROTEINS**

**PROTEINES BIOCIDES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 9050

Publication Year: 1993

15/6/60 (Item 29 from file: 349)

00224457

**BIOCIDAL PROTEINS**

**PROTEINES BIOCIDES**

Publication Language: English

Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 7952  
Publication Year: 1992

15/6/61 (Item 30 from file: 349)

00218464

**BIOCIDAL PROTEINS**

**PROTEINES BIOCIDES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6953

Publication Year: 1992

15/6/62 (Item 31 from file: 349)

00208988

**PROTEINACEOUS ANTI-DENTAL PLAQUE AGENTS**

**AGENTS D'ELIMINATION DE LA PLAQUE DENTAIRE A BASE DE PROTEINES**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 13547

Publication Year: 1992

15/6/63 (Item 32 from file: 349)

00159749

**USE OF THIOREDOXIN, THIOREDOXIN-DERIVED, OR THIOREDOXIN-LIKE DITHIOL  
PEPTIDES IN HAIR CARE PREPARATION**

**UTILISATION DE PEPTIDES DE DITHIOL DE THIOREDOXINE, DERIVES OU ANALOGUES DE  
THIOREDOXINE, DANS DES PREPARATIONS DE SOIN DES CHEVEUX**

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6278

Publication Year: 1989

15/6/64 (Item 1 from file: 5)

0006236844 BIOSIS NO.: 198886076765

**TYROSINE HYDROGEN-BONDING AND ENVIRONMENTAL EFFECTS IN PROTEINS PROBED BY  
UV RESONANCE RAMAN SPECTROSCOPY**

1988

15/6/65 (Item 1 from file: 348)

00642457

**HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN**

**DERIVATE VON ALPHA-HORDOTHIONIN MIT HOHEREM BEHALT AN LYSIN**

**DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE**

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS B                           | (English) | 200137 | 515        |
| CLAIMS B                           | (German)  | 200137 | 492        |
| CLAIMS B                           | (French)  | 200137 | 578        |
| SPEC B                             | (English) | 200137 | 4391       |
| Total word count - document A      |           |        | 0          |
| Total word count - document B      |           |        | 5976       |
| Total word count - documents A + B |           |        | 5976       |

15/6/66 (Item 2 from file: 348)

00579685

**BIOCIDAL PROTEINS**

**BIOZIDE PROTEINE**

**PROTEINES BIOCIDES**

LANGUAGE (Publication,Procedural,Application): English; English; English

**FULLTEXT AVAILABILITY:**

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS B                           | (English) | 200133 | 408        |
| CLAIMS B                           | (German)  | 200133 | 344        |
| CLAIMS B                           | (French)  | 200133 | 431        |
| SPEC B                             | (English) | 200133 | 5803       |
| Total word count - document A      |           |        | 0          |
| Total word count - document B      |           |        | 6986       |
| Total word count - documents A + B |           |        | 6986       |

15/6/67 (Item 3 from file: 348)

00537586

**Natural and synthetic proteins with inhibitory activity towards pathogenic microorganisms.**

**Natürliche und synthetische Proteine mit inhibitorischer Aktivität gegen pathogene Mikroorganismen.**

**Proteines naturelles et synthétiques avec activité inhibitrice contre des microorganismes pathogènes.**

LANGUAGE (Publication,Procedural,Application): English; English; English

**FULLTEXT AVAILABILITY:**

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | EPABF1 | 959        |
| SPEC A                             | (English) | EPABF1 | 5135       |
| Total word count - document A      |           |        | 6094       |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 6094       |

15/6/68 (Item 4 from file: 348)

00245328

**Therapeutic and related uses of dithiol peptides.**

**Therapeutische und verwandte Verwendungen von Dithiol-Peptiden.**

**Utilisations thérapeutiques et apparentées des dithiol peptides.**

LANGUAGE (Publication,Procedural,Application): English; English; English

**FULLTEXT AVAILABILITY:**

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | EPABF1 | 285        |
| SPEC A                             | (English) | EPABF1 | 3897       |
| Total word count - document A      |           |        | 4182       |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 4182       |

15/6/69 (Item 5 from file: 348)

00224186

**Protein-folding enzyme.**

**Protein faltendes Enzym.**

**Enzyme de pliage de protéines.**

LANGUAGE (Publication,Procedural,Application): English; English; English

**FULLTEXT AVAILABILITY:**

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | EPABF1 | 452        |
| SPEC A                             | (English) | EPABF1 | 5235       |
| Total word count - document A      |           |        | 5687       |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 5687       |

15/6/70 (Item 6 from file: 348)

00220520

**Folding disulfide-cross-linkable proteins.**

**In Falten gelegte Proteine, durch Disulfid gebunden.**

**Proteines pliantes liees par pont disulfure en croix.**

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS A                           | (English) | EPABF1 | 515        |
| SPEC A                             | (English) | EPABF1 | 4071       |
| Total word count - document A      |           |        | 4586       |
| Total word count - document B      |           |        | 0          |
| Total word count - documents A + B |           |        | 4586       |

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|         |       |                              |                        |
|---------|-------|------------------------------|------------------------|
| \$0.48  | 0.150 | DialUnits                    | File155                |
| \$0.00  | 1     | Type(s) in Format            | 6                      |
| \$0.00  | 1     | Types                        |                        |
| \$0.48  |       | Estimated cost               | File155                |
| \$6.20  | 1.050 | DialUnits                    | File654                |
| \$6.30  | 9     | Type(s) in Format            | 3                      |
| \$7.50  | 30    | Type(s) in Format            | 6                      |
| \$13.80 | 39    | Types                        |                        |
| \$20.00 |       | Estimated cost               | File654                |
| \$3.61  | 0.287 | DialUnits                    | File399                |
| \$3.61  |       | Estimated cost               | File399                |
| \$1.96  | 0.413 | DialUnits                    | File349                |
| \$8.00  | 32    | Type(s) in Format            | 6                      |
| \$8.00  | 32    | Types                        |                        |
| \$9.96  |       | Estimated cost               | File349                |
| \$4.99  | 0.293 | DialUnits                    | File340                |
| \$4.99  |       | Estimated cost               | File340                |
| \$0.86  | 0.150 | DialUnits                    | File5                  |
| \$0.00  | 1     | Type(s) in Format            | 6                      |
| \$0.00  | 1     | Types                        |                        |
| \$0.86  |       | Estimated cost               | File5                  |
| \$1.55  | 0.341 | DialUnits                    | File348                |
| \$3.40  | 2     | Type(s) in Format            | 3                      |
| \$1.50  | 6     | Type(s) in Format            | 6                      |
| \$4.90  | 8     | Types                        |                        |
| \$6.45  |       | Estimated cost               | File348                |
| \$1.02  | 0.093 | DialUnits                    | File347                |
| \$1.02  |       | Estimated cost               | File347                |
| \$0.45  | 0.120 | DialUnits                    | File65                 |
| \$0.45  |       | Estimated cost               | File65                 |
| \$0.13  | 0.033 | DialUnits                    | File35                 |
| \$0.13  |       | Estimated cost               | File35                 |
| \$3.98  | 0.224 | DialUnits                    | File342                |
| \$3.98  |       | Estimated cost               | File342                |
| \$0.07  | 0.030 | DialUnits                    | File203                |
| \$0.07  |       | Estimated cost               | File203                |
| \$0.24  | 0.045 | DialUnits                    | File156                |
| \$0.24  |       | Estimated cost               | File156                |
| \$0.18  | 0.051 | DialUnits                    | File94                 |
| \$0.18  |       | Estimated cost               | File94                 |
| \$2.81  | 0.090 | DialUnits                    | File398                |
| \$2.81  |       | Estimated cost               | File398                |
| \$0.63  | 0.030 | DialUnits                    | File357                |
| \$0.63  |       | Estimated cost               | File357                |
| \$1.34  | 0.126 | DialUnits                    | File73                 |
| \$1.34  |       | Estimated cost               | File73                 |
| \$0.32  | 0.072 | DialUnits                    | File50                 |
| \$0.32  |       | Estimated cost               | File50                 |
|         |       | OneSearch, 18 files,         | 3.597 DialUnits FileOS |
| \$0.80  |       | TELNET                       |                        |
| \$58.32 |       | Estimated cost this search   |                        |
| \$58.32 |       | Estimated total session cost | 3.597 DialUnits        |

### Status: Signed Off. (3 minutes)

### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)  
Trying 31060000009998...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 04.20.00D

Reconnected in file OS 26feb05 10:16:16

\* \* \*

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1951-2005/Feb W3

(c) format only 2005 The Dialog Corp.

\*File 155: Medline has been reloaded; accession numbers have changed.  
Please see HELP NEWS 154.

File 654:US Pat.Full. 1976-2005/Feb 24

(c) Format only 2005 The Dialog Corp.

File 399:CA SEARCH(R) 1967-2005/UD=14209

(c) 2005 American Chemical Society

\*File 399: Use is subject to the terms of your user/customer agreement.  
Alert feature enhanced for multiple files, etc. See HELP ALERT.

File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210

(c) 2005 WIPO/Univentio

File 340:CLAIMS(R)/US Patent 1950-05/Feb 24

(c) 2005 IFI/CLAIMS(R)

\*File 340: 2004 Reload is online as of October 6, 2004. Pricing  
changes effective October 1, 2004. See HELP NEWS 340 for details.

File 5:Biosis Previews(R) 1969-2005/Feb W3

(c) 2005 BIOSIS

\*File 5: Price change effective Jan 1, 2005. Enter HELP  
RATES 5 for details.

File 348:EUROPEAN PATENTS 1978-2005/Feb W03

(c) 2005 European Patent Office

File 347:JAPIO Nov 1976-2004/Oct(Updated 050208)

(c) 2005 JPO & JAPIO

\*File 347: JAPIO data problems with year 2000 records are now fixed.  
Alerts have been run. See HELP NEWS 347 for details.

File 65:Inside Conferences 1993-2005/Feb W3

(c) 2005 BLDSC all rts. reserv.

File 35:Dissertation Abs Online 1861-2005/Feb

(c) 2005 ProQuest Info&Learning

File 342:Derwent Patents Citation Indx 1978-05/200510

(c) 2005 Thomson Derwent

File 203:AGRIS. 1974-2004/Nov

Dist by NAL, Intl Copr. All rights reserved

File 156:ToxFile 1965-2005/Feb W3

(c) format only 2005 The Dialog Corporation

\*File 156: Updating of ToxFile has resumed, with  
UD=20041205.

File 94:JICST-EPlus 1985-2005/Jan W2

(c)2005 Japan Science and Tech Corp(JST)



File 398:Chemsearch 1957-2005/Jan

(c) 2005 Amer.Chem.Soc.

\*File 398: Use is subject to the terms of your user/customer agreement.  
Problems with SORT. RANK charge added. See HELP RATES 398.

File 357:Derwent Biotech Res. 1982-2005/Feb W4

(c) 2005 Thomson Derwent & ISI

File 73:EMBASE 1974-2005/Feb W3

(c) 2005 Elsevier Science B.V.

\*File 73: Price change effective Jan 1, 2005. Enter HELP  
RATES 73 for details.

File 50:CAB Abstracts 1972-2005/Jan

(c) 2005 CAB International

Set Items Description

--- -----

Cost is in DialUnits

?ds

| Set | Items  | Description                                               |
|-----|--------|-----------------------------------------------------------|
| S1  | 373    | ALPHA (2N) THIONIN?                                       |
| S2  | 313    | RD (unique items)                                         |
| S3  | 279736 | 'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION' |
| S4  | 53755  | 'EDTA'                                                    |
| S5  | 44607  | R1-R4                                                     |
| S6  | 79     | S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)      |
| S7  | 51     | S6/2002:2005                                              |
| S8  | 28     | S6 NOT S7                                                 |
| S9  | 689    | E3-E33                                                    |
| S10 | 1      | 'PUROTHIORINS'                                            |
| S11 | 689    | S9 OR S10                                                 |
| S12 | 128    | S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)  |
| S13 | 50     | S12/2002:2005                                             |
| S14 | 78     | S12 NOT S13                                               |
| S15 | 70     | S14 NOT S8                                                |

?t s15/9/1

15/9/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2005 The Dialog Corp. All rts. reserv.

08856644 PMID: 2610351

**Histochemical localization of cysteine-rich proteins by tissue printing on nitrocellulose.**

Pont-Lezica R F; Varner J E

Department of Biology, Washington University, St. Louis, Missouri 63130.

Analytical biochemistry (UNITED STATES) Nov 1 1989, 182 (2) p334-7,

ISSN 0003-2697 Journal Code: 0370535

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A rapid technique for the histochemical localization of cysteine-rich proteins in plant tissues was developed. It is based on the immediate transfer of proteins to nitrocellulose membranes when a fresh cut organ is pressed against the membrane surface. The print was labeled for cysteine-rich proteins by reduction and alkylation of cysteinyl residues with dansylated iodoacetamide [N-iodoacetyl-N'-(-5-sulfo-1-naphthyl) ethylenediamine ]. The S-carboxymethylated proteins were visualized by their fluorescence when excited with 360 nm light.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.

Descriptors: \*Collodion; \*Cysteine--analysis--AN; \*Histocytochemistry  
--methods--MT; \*Proteins--analysis--AN; Hordeum--analysis--AN; Lectins  
--analysis--AN; Membranes, Artificial; Naphthalenesulfonates;  
Oxidation-Reduction; Plant Lectins; Plant Proteins--analysis--AN; Potatoes

--analysis--AN

CAS Registry No.: 0 (Lectins); 0 (Naphthalenesulfonates); 0 (Plant Lectins); 0 (Plant Proteins); 0 (Proteins); 36930-63-9 (1,5-I-AEDANS); 52-90-4 (Cysteine); 9004-70-0 (Collodion); 9009-72-7 (purothionin)

Record Date Created: 19900222

Record Date Completed: 19900222

?t s15/3,kwic/2-70

>>>KWIC option is not available in file(s): 398, 399

15/3,KWIC/2 (Item 1 from file: 654)

DIALOG(R)File 654:US Pat.Full.

(c) Format only 2005 The Dialog Corp. All rts. reserv.

0004925643

Derwent Accession: 2002-010726

**Compositions and methods for identifying and targeting cancer cells of alimentary canal origin**

Inventor: Scott Waldman, INV

Jason Park, INV

Stephanie Schulz, INV

Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ,

MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia, PA, 19103, US

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 20010036635        | A1   | 20011101 | US 2001819247         | 20010327       |
| Provisional |                       |      |          | US 60-192229          | 20000327       |

Fulltext Word Count: 30220

Summary of the Invention:

...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein- specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine**-tetraacetic acid ( **EDTA** ). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):

...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/3 (Item 2 from file: 654)

DIALOG(R) File 654:US Pat.Full.

(c) Format only 2005 The Dialog Corp. All rts. reserv.

0004918035

Derwent Accession: 2002-381264

**Compositions and methods for identifying and targeting cancer cells of alimentary canal origin**

Inventor: Scott Waldman, INV

Jason Park, INV

Stephanie Schulz, INV

Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ,  
MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia,  
PA, 19103, US

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 20010029019        | A1   | 20011011 | US 2001819249         | 20010327       |
| Provisional |                       |      |          | US 60-192229          | 20000327       |

Fulltext Word Count: 30022

**Summary of the Invention:**

...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine**-tetraacetic acid ( **EDTA** ). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

**Non-exemplary or Dependent Claim(s):**

...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/4 (Item 3 from file: 654)

DIALOG(R) File 654:US Pat.Full.

(c) Format only 2005 The Dialog Corp. All rts. reserv.

0004903757 \*\*IMAGE Available

Derwent Accession: 1993-100978

**Biocidal proteins**

Inventor: Willem Broekaert, INV

Bruno Cammue, INV

**CERTIFICATE OF CORRECTION**

**C/ Methods of identifying and detecting pancreatic cancer  
; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN  
TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS,  
PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS  
A TUMOR**

Inventor: Weinberg, David, Philadelphia, PA  
Waldman, Scott A., Ardmore, PA  
Barber, Michael T., Paoli, PA  
Biswas, Sanjoy, Philadelphia, PA  
Assignee: Thomas Jefferson University(02), Philadelphia, PA  
Jefferson, Thomas University (Code: 06943)  
Examiner: Eyler, Yvonne (Art Unit: 162)  
Assistant Examiner: Holleran, Anne L.  
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6187536            | A    | 20010213 | US 9825534            | 19980218       |
| Provisional |                       |      |          | US 60-38063           | 19970218       |

Fulltext Word Count: 23168

**Summary of the Invention:**

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic acid** (DTPA) or **ethylenediamine**-tetraacetic acid ( **EDTA** ). One skilled in the art would readily recognize other fluorescence-emitting metals as well as ...

15/3,KWIC/8 (Item 7 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

4427576  
Derwent Accession: 2000-687452

**Utility**

**C/ X-ray guided drug delivery  
; TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION**

Inventor: Hallahan, Dennis E., Nashville, TN  
Assignee: Vanderbilt University(02), Nashville, TN  
Vanderbilt University (Code: 88418)  
Examiner: Schwartzman, Robert A. (Art Unit: 166)  
Assistant Examiner: Sandals, William  
Law Firm: Jenkins & Wilson, P.A.

|  | Publication<br>Number | Kind | Date | Application<br>Number | Filing<br>Date |
|--|-----------------------|------|------|-----------------------|----------------|
|--|-----------------------|------|------|-----------------------|----------------|

-----  
Main Patent    US 6159443            A    20001212    US 99302456            19990429

Fulltext Word Count: 26146

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...

Description of the Invention:

...by staining for factor VIII. Confluent cells were harvested with 0.1% collagenase 0.01% **EDTA** and subcultured at a ratio of 1:3. HUVECs were used at third passage;

Non-exemplary or Dependent Claim(s):

...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...daunorubicin, cytosine arabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cyclophosphamide, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...

15/3,KWIC/9            (Item 8 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

4384858

Derwent Accession: 1999-180474

Utility

C/ Compositions that specifically bind to colorectal cancer cells and methods of using the same  
; IN VITRO METHOD OF DIAGNOSING METASTASIZED COLORECTAL CANCER BY DETECTING GENE EXPRESSION OF COLORECTAL CANCER-ASSOCIATED TRANSCRIPT-1, AN ALTERNATE FORM OF HEAT-STABLE TOXIN RECEPTOR, IN CELLS OF SAMPLE

Inventor: Waldman, Scott A., Ardmore, PA  
          Pearlman, Joshua M., Philadelphia, PA  
          Barber, Michael T., Paoli, PA  
          Schulz, Stephanie, West Chester, PA  
          Parkinson, Scott J., Philadelphia, PA  
Assignee: Thomas Jefferson University(02), Philadelphia, PA  
          Jefferson, Thomas University (Code: 06943)  
Examiner: Eyler, Yvonne (Art Unit: 162)  
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6120995            | A    | 20000919 | US 97908643           | 19970807       |

Fulltext Word Count: 34307

Description of the Invention:

...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses,

2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...hours at room temperature in 0.4M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and... activity of the ST peptide. [<sup>sup</sup>]111 In is rapidly and potently chelated by either **EDTA** ( **ethylenediaminetetraacetic** acid) or DTPA ( **diethylenetriaminepentaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [<sup>sup</sup>]111 In-DTPA is ...

Non-exemplary or Dependent Claim(s):

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/16 (Item 15 from file: 654)  
 DIALOG(R)File 654:US Pat.Full.  
 (c) Format only 2005 The Dialog Corp. All rts. reserv.

4085182  
 Derwent Accession: 1997-480228

Utility

C/ Alteration of amino acid compositions in seeds

Inventor: Jung, Rudolf, Des Moines, IA  
 Hastings, Craig, Perry, IA  
 Coughlan, Sean, Des Moines, IA  
 Hu, David, Johnston, IA  
 Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA  
 Pioneer Hi-Bred International Inc (Code: 17947)  
 Examiner: LeGuyader, John (Art Unit: 165)  
 Assistant Examiner: McGarry, Sean  
 Law Firm: Pioneer Hi-Bred International, Inc.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 5850016            | A    | 19981215 | US 96618911           | 19960320       |

Fulltext Word Count: 12377

Description of the Invention:

...invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (1976); incorporated herein in its... pH 5.2 and concentrated in the dialysis bags to about 100 ml with dry **polyethyleneglycol** (PEG 8000). Precipitated contaminating globulin proteins are removed by centrifugation at 6000Xg for 15 min...

15/3,KWIC/17 (Item 16 from file: 654)  
 DIALOG(R)File 654:US Pat.Full.  
 (c) Format only 2005 The Dialog Corp. All rts. reserv.

4057665

TES 5 for details.

File 34:SciSearch(R) Cited Ref Sci 1990-2005/Feb W3

(c) 2005 Inst for Sci Info

\*File 34: Price change effective Jan 1, 2005. Enter HELP  
RATES 34 for details.

File 35:Dissertation Abs Online 1861-2005/Jan

(c) 2005 ProQuest Info&Learning

File 48:SPORTDiscus 1962-2005/May

(c) 2005 Sport Information Resource Centre

File 65:Inside Conferences 1993-2005/Feb W3

(c) 2005 BLDSC all rts. reserv.

File 71:ELSEVIER BIOBASE 1994-2005/Feb W2

(c) 2005 Elsevier Science B.V.

File 73:EMBASE 1974-2005/Feb W3

(c) 2005 Elsevier Science B.V.

\*File 73: Price change effective Jan 1, 2005. Enter HELP  
RATES 73 for details.

File 91:MANTIS(TM) 1880-2005/Feb

2001 (c) Action Potential

File 94:JICST-EPlus 1985-2005/Jan W2

(c)2005 Japan Science and Tech Corp(JST)

File 98:General Sci Abs/Full-Text 1984-2004/Dec

(c) 2005 The HW Wilson Co.

File 135:NewsRx Weekly Reports 1995-2005/Feb W3

(c) 2005 NewsRx

\*File 135: New newsletters are now added. See Help News135 for the  
complete list of newsletters.

File 144:Pascal 1973-2005/Feb W2

(c) 2005 INIST/CNRS

\*File 144: Price change effective Jan 1, 2005. Enter HELP  
RATES 144 for details.

File 149:TGG Health&Wellness DB(SM) 1976-2005/Feb W2

(c) 2005 The Gale Group

File 156:ToxFile 1965-2005/Feb W3

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\*File 156: Updating of ToxFile has resumed, with  
UD=20041205.

File 159:Cancerlit 1975-2002/Oct

(c) format only 2002 Dialog Corporation

\*File 159: Cancerlit is no longer updating.

Please see HELP NEWS159.

File 162:Global Health 1983-2005/Jan

(c) 2005 CAB International

File 164:Allied & Complementary Medicine 1984-2005/Feb

(c) 2005 BLHCIS

File 172:EMBASE Alert 2005/Feb W2

(c) 2005 Elsevier Science B.V.

\*File 172: Price change effective Jan 1, 2005. Enter HELP  
RATES 172 for details.

File 266:FEDRIP 2004/Nov

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File 369:New Scientist 1994-2005/Feb W2

(c) 2005 Reed Business Information Ltd.

File 370:Science 1996-1999/Jul W3

(c) 1999 AAAS

\*File 370: This file is closed (no updates). Use File 47 for more current  
information.

File 399:CA SEARCH(R) 1967-2005/UD=14209

(c) 2005 American Chemical Society

\*File 399: Use is subject to the terms of your user/customer agreement.

Alert feature enhanced for multiple files, etc. See HELP ALERT.

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

\*File 434: Price change effective Jan 1, 2005. Enter HELP  
RATES 434 for details.

File 444:New England Journal of Med. 1985-2005/Feb W3

(c) 2005 Mass. Med. Soc.

Set Items Description

Cost is in DialUnits  
?ds

| Set | Items  | Description                                             |
|-----|--------|---------------------------------------------------------|
| S1  | 369    | 'THIONIN'                                               |
| S2  | 21702  | 'EDTA'                                                  |
| S3  | 2176   | 'ETHYLENEDIAMINES' OR 'ETHYLENEDIAMINETETRAACETIC ACID' |
| S4  | 2173   | R1-R2                                                   |
| S5  | 2      | S1 AND (S2 OR S3 OR S4)                                 |
| S6  | 211502 | EDTA? OR ETHYLENEDIAMINE?                               |
| S7  | 7121   | THIONIN?                                                |
| S8  | 77     | S6 AND S7                                               |
| S9  | 56     | RD (unique items)                                       |
| S10 | 5      | S9/2000:2005                                            |
| S11 | 51     | S9 NOT S10                                              |

?t s11/9/1 2 3 4 5 8 7 10 11 13 14 22 51

11/9/1 (Item 1 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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12120265 PMID: 9421187

**Mechanisms by which thionin induces susceptibility of S49 cell membranes to extracellular phospholipase A2.**

Wilson H A; Huang W; Waldrip J B; Judd A M; Vernon L P; Bell J D

Department of Zoology, Brigham Young University, Provo, UT 84602, USA.

Biochimica et biophysica acta (NETHERLANDS) Nov 15 1997, 1349 (2)  
p142-56, ISSN 0006-3002 Journal Code: 0217513

Contract/Grant No.: GM-49710; GM; NIGMS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Whereas cells normally resist attack by PLA2, they become susceptible under certain pathological conditions. To ascertain the regulatory mechanisms that induce cellular susceptibility to PLA2, the effect of **thionin** on S49 cells was examined in the presence of PLA2. **Thionin** alone was unable to evoke hydrolysis of the lipid bilayer. Likewise, the addition of PLA2 alone caused production of only a minimal amount of free fatty acid. However, **thionin** and PLA2 together resulted in significant hydrolysis of the cell membrane. **Thionin** caused perturbation of the bilayer structure as suggested by the changes in the emission spectra of laurdan and the permeability of the membrane to propidium iodide. These changes correlated quantitatively with the susceptibility of the lipid bilayer to PLA2. Furthermore, **thionin** induced a modest increase in intracellular Ca2+. The source of this Ca2+ was the extracellular fluid since **EDTA** in the extracellular medium inhibited the Ca2+ influx. Moreover, cobalt chloride, a universal Ca2+ channel blocker, prevented the rise in intracellular Ca2+, the uptake of propidium iodide, and the susceptibility to PLA2 induced by **thionin**. In contrast, the changes in the laurdan emission caused by the **thionin** were not affected by the cobalt. Furthermore, incubation of the cells with the calcium ionophore A23187 also caused the cells to become susceptible to PLA2. We hypothesize that **thionin** causes S49 cell membranes to become susceptible to PLA2 by a Ca2+-dependent perturbation of the bilayer structure.

Tags: Research Support, U.S. Gov't, P.H.S.

Descriptors: \*Phenothiazines--pharmacology--PD; \*Phospholipases A  
--pharmacology--PD; Animals; Arachidonic Acid--metabolism--ME; Calcimycin  
--pharmacology--PD; Calcium--metabolism--ME; Cell Membrane--metabolism--ME;



Lipid Bilayers--metabolism--ME; Lymphoma--metabolism--ME; Mice; Tumor Cells, Cultured

CAS Registry No.: 0 (Lipid Bilayers); 0 (Phenothiazines); 506-32-1 (Arachidonic Acid); 52665-69-7 (Calcimycin); 581-64-6 (thionine); 7440-70-2 (Calcium)

Enzyme No.: EC 3.1.1.- (Phospholipases A)

Record Date Created: 19980115

Record Date Completed: 19980115

11/9/2 (Item 2 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10106631 PMID: 7680580

**Thionin staining of paraffin and plastic embedded sections of cartilage.**

Bulstra S K; Drukker J; Kuijer R; Buurman W A; van der Linden A J

Department of Orthopaedic Surgery, University Hospital Maastricht, State University of Limburg, The Netherlands.

Biotechnic & histochemistry - official publication of the Biological Stain Commission (UNITED STATES) Jan 1993, 68 (1) p20-8, ISSN 1052-0295 Journal Code: 9107378

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.

Tags: Comparative Study

Descriptors: \*Cartilage, Articular--cytology--CY; \*Methacrylates; \*Paraffin Embedding; \*Phenothiazines--metabolism--ME; Aged; Cartilage, Articular--anatomy and histology--AH; Chondroitin Lyases--metabolism--ME; Decalcification Technique; Glycosaminoglycans--metabolism--ME; Humans; Phenazines; Plastic Embedding; Sensitivity and Specificity; Staining and Labeling--methods--MT

CAS Registry No.: 0 (Glycosaminoglycans); 0 (Methacrylates); 0 (Phenazines); 0 (Phenothiazines); 477-73-6 (safranin T); 581-64-6 (thionine); 868-77-9 (hydroxyethyl methacrylate)

Enzyme No.: EC 4.2.2.- (Chondroitin Lyases)

Record Date Created: 19930412

Record Date Completed: 19930412

11/9/3 (Item 3 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

(c) format only 2005 The Dialog Corp. All rts. reserv.

05131256 PMID: 594487

**[Selective photooxidation of methionine, tyrosine and tryptophane using thionine and toluidine blue as sensitizers (author's transl)]**

Fotooxidacion selectiva de metionina, tirosina y triptofano utilizando tionina y azul de toluidina como fotosensibilizadores.

Iborra J L; Llorca F I; Pastor R F; Garcia J V

Revista espanola de fisiologia (SPAIN) Dec 1977, 33 (4) p297-304,  
ISSN 0034-9402 Journal Code: 0404475

Publishing Model Print

Document type: Journal Article ; English Abstract

Languages: SPANISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Thionine and toluidine blue were used as sentizers on photooxidation processes of methionine, tryosine and tryptophane. They were more effective than methylene blue. Methionine was photooxidized to sulfoxide and tryptophane to kinurenine. A tyrosine-sensitizer addition compound was postulated. Dye concentration, pH, temperature and EDTA presence conditions were determined on each one of the modification reactions. Methionine at acid pH was selectively modified. On the basis of obtained results and published references, a direct interaction of singlet oxygen with methionine and tryptophase and the excited dye with tyrosine was respectively discussed.

Tags: Comparative Study

Descriptors: \*Amino Acids--metabolism--ME; \*Photosynthesis--drug effects--DE; \*Tyrosine--metabolism--ME; Ergothioneine--pharmacology--PD; Methionine--metabolism--ME; Oxidation-Reduction--drug effects--DE; Photic Stimulation; Tolonium Chloride--pharmacology--PD; Tryptophan--metabolism--ME

CAS Registry No.: 0 (Amino Acids); 497-30-3 (Ergothioneine); 55520-40-6 (Tyrosine); 63-68-3 (Methionine); 73-22-3 (Tryptophan); 92-31-9 (Tolonium Chloride)

Record Date Created: 19780223

Record Date Completed: 19780223

11/9/4 (Item 4 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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04488011 PMID: 806084

**Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis III. Consequences of a newly found pKT of thionine on the mechanism in basic solutions.**

Bonneau R; Pereyre J

Photochemistry and photobiology (ENGLAND) Mar 1975, 21 (3) p173-7,  
ISSN 0031-8655 Journal Code: 0376425

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Descriptors: \*Dyes; \*Edetic Acid; \*Light; \*Oxidation-Reduction; \*Phenothiazines; \*Photolysis; \*Thiazines--metabolism--ME; Imines

CAS Registry No.: 0 (Dyes); 0 (Imines); 0 (Phenothiazines); 0 (Thiazines); 60-00-4 (Edetic Acid)

Record Date Created: 19750829

Record Date Completed: 19750829

11/9/5 (Item 1 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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0003236407 BIOSIS NO.: 198171055366

**CHARACTERIZATION OF THE RED LIGHT INDUCED REDUCTION OF A PARTICLE**

**ASSOCIATED B TYPE CYTOCHROME FROM CORN IN THE PRESENCE OF METHYLENE BLUE**

AUTHOR: WIDELL S (Reprint); BRITZ S J; BRIGGS W R

AUTHOR ADDRESS: DEP PLANT PHYSIOL, BOX 7007, S-22007, LUND 7, SWEDEN\*\*

SWEDEN

JOURNAL: Photochemistry and Photobiology 32 (5): p669-678 1980

ISSN: 0031-8655

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

**ABSTRACT:** Methylene blue transfers electrons to a membrane-associated b-type cytochrome in particulate fractions from corn coleoptiles. The  $K_m$  for methylene blue is less than 1  $\mu M$  under optimal conditions. This reaction is destroyed by boiling, but not by 7 M urea. Kinetic analyses of the influence of light intensity on cytochrome reduction suggest that a 1st order photochemical reaction is limiting. Free **EDTA** may serve as an electron donor in this system at least at high methylene blue and protein concentrations. The photoactivity does not coincide either with mitochondrial or endoplasmic reticulum markers, and may be localized in plasma membrane. There is an estimated 5  $\times 10^{-10}$  mol photoreducible cytochrome/g coleoptile tissue. Studies on the effect of pH on the reaction in the presence of methylene blue or **thionine** indicate that dye photoreduction itself is not rate-limiting. Wavelength dependence studies suggest that it is methylene blue monomer and not dimer which mediates the reaction. Although  $O_2$  is apparently required for the reaction, neither superoxide nor excited singlet oxygen appear to be involved. The reaction mechanism is still unknown.

REGISTRY NUMBERS: 61-73-4: METHYLENE BLUE; 60-00-4: **EDTA** ; 7782-44-7: OXYGEN

DESCRIPTORS: COLEOPTILES ELECTRON TRANSFER **EDTA** WAVELENGTH DEPENDENCE OXYGEN REQUIREMENT

**DESCRIPTORS:**

MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics;  
Enzymology--Biochemistry and Molecular Biophysics; Radiation Biology  
BIOSYSTEMATIC NAMES: Gramineae--Monocotyledones, Angiospermae,  
Spermatophyta, Plantae

COMMON TAXONOMIC TERMS: Angiosperms; Monocots; Plants; Spermatophytes;  
Vascular Plants

CHEMICALS & BIOCHEMICALS: METHYLENE BLUE; **EDTA** ; OXYGEN

**CONCEPT CODES:**

10012 Biochemistry - Gases  
10060 Biochemistry studies - General  
10064 Biochemistry studies - Proteins, peptides and amino acids  
10065 Biochemistry studies - Porphyrins and bile pigments  
10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation  
10604 External effects - Light and darkness  
10802 Enzymes - General and comparative studies: coenzymes  
51516 Plant physiology - Light and radiation effects  
52504 Agronomy - Grain crops

**BIOSYSTEMATIC CODES:**

25305 Gramineae

11/9/8 (Item 3 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2005 Inst for Sci Info. All rts. reserv.

01233933 Genuine Article#: GG799 Number of References: 91

Title: MODEL PHOTO GALVANIC SYSTEMS FOR THE CONVERSION OF SOLAR-ENERGY

Author(s): SHAGISULTANOVA GA; TAITIS AM; TIMONOV AM

Corporate Source: AI GERTSEN STATE TEACHERS INST/LENINGRAD//USSR/

Journal: JOURNAL OF APPLIED CHEMISTRY OF THE USSR, 1990, V63, N12, P  
2427-2440

Language: ENGLISH Document Type: ARTICLE

Geographic Location: UNION OF SOVIET SOCIALIST REPUBLICS

Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth  
Sciences

Journal Subject Category: CHEMISTRY, APPLIED

Identifiers--KeyWords Plus: THIONINE -COATED ELECTRODE;

ETHYLENEDIAMINETETRAACETIC ACID SYSTEM; PHOTO-CHEMICAL MECHANISMS;  
PHOTOELECTROCHEMICAL CELL; METHYLENE-BLUE; RATE CONSTANT; IRON SYSTEM;  
POWER; EFFICIENCY; KINETICS

Research Fronts: 89-6804 002 (PHOTO GALVANIC CELL FOR SOLAR-ENERGY  
CONVERSION; PHENOSAFRANIN DYE; METHYLENE BLUE-NITRILOTRIACETIC ACID  
SYSTEM)

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11/9/7 (Item 2 from file: 34)

DIALOG(R) File 34:SciSearch(R) Cited Ref Sci  
 (c) 2005 Inst for Sci Info. All rts. reserv.

02513289 Genuine Article#: LG689 Number of References: 0  
 (NO REFS KEYED)

Title: USE OF TRITON X-100 IN A PHOTOGALVANIC CELL FOR SOLAR-ENERGY  
 CONVERSION AND STORAGE - THIONINE EDTA SYSTEM

Author(s): AMETA SC; CHITTORA AK; KHAMESRA S; AMETA R

Corporate Source: SUKHADIA UNIV, UNIV COLL SCI, DEPT CHEM, 15 RADHEYSHYAM  
 ST, BHARAMPOLE GATE/UDAIPUR 313001//INDIA/

Journal: ARABIAN JOURNAL FOR SCIENCE AND ENGINEERING, 1992, V17, N4A (OCT)  
 , P477-480

ISSN: 0377-9211

Language: ENGLISH Document Type: ARTICLE

Geographic Location: INDIA

Subfile: SciSearch; CC ENGI--Current Contents, Engineering, Technology &  
 Applied Sciences

Journal Subject Category: MULTIDISCIPLINARY SCIENCES

Abstract: Thionine , ethylenediaminetetraacetic acid, and Triton X-100  
 (Tx) have been used as photosensitizer, reductant, and surfactant,  
 respectively in a photogalvanic cell for solar energy conversion. The

photocurrent and photopotential generated by this cell were 70.0  $\mu$ A and 888.0 mV, respectively. The effect of various parameters on the electrical output of the cell was studied, and current-voltage characteristics of the cell have also been observed.

11/9/10 (Item 1 from file: 144)  
DIALOG(R) File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

08896137 PASCAL No.: 90-0064116

**Use of the thionine - EDTA system in photogalvanic cells for solar energy conversion**

AMETA S C; SADHNA KHAMESRA; SUSHILA LODHA; RAMESHWER AMETA  
Sukhadia univ., dep. chemistry, Udaipur 313 001, India  
Journal: Journal of photochemistry and photobiology. A. Chemistry, 1989  
, 48 (1) 81-86

ISSN: 531731 CODEN: JPPCEJ Availability: CNRS-15990A

No. of Refs.: 19 ref.

Document Type: P (Serial) ; A (Analytic)

Country of Publication: Switzerland

Language: English

Description d'une cellule solaire utilisant de la **thionine** comme photosensibilisant et l' **EDTA** comme reducteur: caracteristiques potentiometriques en fonction de la concentration en **thionine** , en **EDTA** , du pH et de la geometrie de la couche et mecanismes de reaction

English Descriptors: Solar cell; Energy conversion; Electrical characteristic; Photosensitizer; Medium effect; Potentiometry; Chemical concentration; Experimental study; Reaction mechanism; **EDTA**

French Descriptors: Cellule solaire; Conversion energie; Caracteristique electrique; Photosensibilisant; Effet milieu; Potentiometrie; Concentration chimique; Etude experimentale; Mecanisme reaction; **EDTA** ; **Thionine**

Classification Codes: 230C02B; 001D06C02

11/9/11 (Item 2 from file: 144)  
DIALOG(R) File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

05746232 PASCAL No.: 84-0247280

**Electrochemistry and photoelectrochemistry of dye-incorporated clay-modified electrode**

KAMAT P V

Univ. Notre Dame, radiation lab., Notre Dame IN 46556, USA

Journal: Journal of electroanalytical chemistry and interfacial electrochemistry, 1984, 163 (1-2) 389-394

ISSN: 0022-0728 Availability: CNRS-1150

No. of Refs.: 13 ref.

Document Type: P (Serial) ; A (Analytic)

Country of Publication: Switzerland

Language: English

Etudes dans le cas de **thionine** incorporee dans des couches minces d'argile fixees sur des electrodes de SnO SUB 2 et de Pt. Resultats obtenus par etudes de voltammetrie cyclique, ainsi que donnees sur la generation de l'effet photoelectrochimique sous l'effet d'un rayonnement de  $\lambda > 460$  nm et en milieu aqueux contenant de l' **EDTA** 0,2 M

English Descriptors: Photoelectrochemistry; Semiconductor materials; Electrodes; Transition metal; Metal Oxides; Platinum; Thin layer electrode; Modified material; Chemical uptake; Thiazine dye; Electrochemical reaction; Cyclic voltammetry; Photoelectrochemical reaction; Aqueous solution; Organic solvent; Tin IV Oxides; Clay

French Descriptors: Photoelectrochimie; Semiconducteur; Electrode; Metal transition; Metal Oxyde; Platine-ACT; Electrode couche mince; Matériau modifié; Fixation chimique; Colorant thiazinique; Reaction electrochimique; Voltammétrie cyclique; Reaction photoelectrochimique; Solution aqueuse; Solvant organique; Etain IV Oxyde-ACT; Argile; **Thionine** ; Violet Lauth; **EDTA** -SUB

Classification Codes: 001C01H06

11/9/13 (Item 4 from file: 144)  
DIALOG(R) File 144:Pascal  
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04862032 PASCAL No.: 83-0108558  
**Effect of DNA and other polyanions on the EDTA induced photoreduction of thionine**  
MEDINI KANTA PAL; MANNA P C  
Univ. Kalyani, dep. chemistry, Kalyani 741235, India  
Journal: Makromol. Chem., 1982, 183 (11) 2811-2821  
ISSN: 0025-116X Availability: CNRS-4111  
No. of Refs.: 12 ref.  
Document Type: P (Serial) ; A (Analytic)  
Country of Publication: Switzerland  
Language: English  
Influence de l'ADN, de l'ARN et du polystyrenesulfonate

English Descriptors: DNA; Polyanion; Reduction; Photochemical reaction; Styrenesulfonate polymer; RNA; Basic dye

French Descriptors: DNA; Polyanion; Reduction; Reaction photochimique; Styrenesulfonate polymère; RNA; Colorant basique; **Thionine** -ENT; Tétracétique acide( **ethylenediamine** ); Utilisation

Classification Codes: 780A02E

11/9/14 (Item 5 from file: 144)  
DIALOG(R) File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

04003372 PASCAL No.: 75-0028838  
**MECHANISM OF PHOTOREDUCTION OF THIAZINE DYES BY EDTA STUDIED BY FLASH PHOTOLYSIS. III. CONSEQUENCES OF A NEWLY FOUND PK SUB T OF THIONINE ON THE MECHANISM IN BASIC SOLUTIONS**  
BONNEAU R; PEREYRE J  
LAB. CHIM. PHYS. A, UNIV. BORDEAUX I, 33405 TALENCE, FRANCE  
Journal: PHOTOCHEM. AND PHOTOBIOLOG., 1975, 21 (3) 173-177  
Availability: CNRS-9410  
No. of Refs.: 13 REF.  
Document Type: P (SERIAL) ; A (ANALYTIC)  
Country of Publication: UNITED KINGDOM  
Language: ENGLISH  
EQUILIBRE ENTRE LES FORMES NEUTRE ET MONOCATIONIQUE DE LA **THIONINE** TRIPLET, SUP 3 T ET SUP 3 TH SUP + (PK SUB T =8,9). PAS D'EQUILIBRE IDENTIQUE AVEC LE BLEU DE METHYLENE. DIFFERENCE DUE A LEUR PHOTOREDUCTION DIFFERENTE PAR **EDTA** . LE RENDEMENT QUANTIQUE DE FORMATION DU COLORANT SEMI REDUIT EST PROPORTIONNEL AU RENDEMENT DE REDUCTION GLOBAL. MECANISMES

English Descriptors: METHYLENE BLUE; ORGANIC DYE; MONOCYCLIC COMPOUND; ACIDITY CONSTANT; **EDTA** ; SULFUR NITROGEN HETEROCYCLE; REACTION MECHANISM ; FLASH PHOTOLYSIS; PHOTOCHEMICAL REACTION; REDUCTION; QUANTUM YIELD  
English Generic Descriptors: PHYSICAL CHEMISTRY; PHOTOCHEMISTRY

French Descriptors: COLORANT ORGANIQUE; THIAZINE; BLEU METHYLENE; **THIONINE** ; HETEROCYCLE SOUFRE AZOTE; COMPOSE MONOCYCLIQUE; **EDTA** ; REDUCTION;

REACTION PHOTOCHEMIQUE; CONSTANTE ACIDITE; RENDEMENT QUANTIQUE; PHOTOLYSE  
ECLAIR; MECANISME REACTION  
French Generic Descriptors: CHIMIE PHYSIQUE; PHOTOCHEMIE

Classification Codes: 170A14B02A

11/9/22 (Item 13 from file: 144)  
DIALOG(R) File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

00112095 PASCAL No.: 73-0032420  
INDICADORES FOTO-REDOX EN AMPEROMETRIA. DETERMINACIONES INDIRECTAS DE  
ANIONES CON ETILENODIAMINO TETRAACETO DIHYDROGENO DISODICO  
(INDICATEURS PHOTO-REDOX EN AMPEROMETRIE. DOSAGE INDIRECT DES ANIONS AVEC  
L' EDTA -H SUB 2 NA SUB 2 )  
SIERRA F; SANCHEZ-PEDRENO C; PEREZ RUIZ T; MARTINEZ LOZANO C  
C.S.I.C., DEP. QUIM. ANAL., DE MURCIA  
Journal: INFORM. QUIM. ANAL., 1973, 27 (2) 93-98  
Availability: CNRS-2711  
No. of Refs.: 6 REF.  
Document Type: P (SERIAL)  
Country of Publication: SPAIN  
Language: SPANISH Summary Language: ENGLISH  
EMPLOI DE **THIONINE** COMME INDICATEUR REDOX-PHOTOCHEMIQUE POUR LE TITRAGE  
INDIRECT DE CRO SUB 4 SUP 2- , SO SUB 4 SUP 2- , PO SUB 4 H SUB 2 SUP - ET  
(FE(CN) SUB 6 ) SUP 4-

English Descriptors: AMPEROMETRY; CHEMICAL ANALYSIS; CHEMICAL COMPOSITION;  
CHROMATES; ANIONIC COMPLEX; **EDTA** ; ANALYTICAL INDICATOR; REDOX INDICATOR  
; PHOSPHATES; ABSORPTION SPECTROMETRY; ABSORPTION SPECTROSCOPY; SULFATES  
English Generic Descriptors: ANALYTICAL CHEMISTRY  
French Descriptors: **THIONINE** ; INDICATEUR ANALYTIQUE; CHROMATE; SULFATE;  
PHOSPHATE; FER II COMPOSE; FER II COMILEXE; COMPLEXE CYANO; COMPLEXE  
ANIONIQUE; ANALYSE CHIMIQUE; AMPEROMETRIE; TITRAGE AMPEROMETRIQUE; **EDTA**  
; INDICATEUR REDOX; SPECTROMETRIE ABSORPTION; ANALYSE PAR; METHODE  
INDIRECTE; CYANO; ANALYSE  
French Generic Descriptors: CHIMIE ANALYTIQUE

Classification Codes: 170C05B06

11/9/51 (Item 3 from file: 434)  
DIALOG(R) File 434:SciSearch(R) Cited Ref Sci  
(c) 1998 Inst for Sci Info. All rts. reserv.

01388440 Genuine Article#: CW889 Number of References: 20  
Title: DETERMINATION OF IODIDE TRACES USING INHIBITORY EFFECT IN  
PHOTOCHEMICAL INTERACTION OF THIONINE AND ETHYLENEDIAMINETETRAACETIC  
ACID  
Author(s): SIERRA F; SANCHEZPEDRENO C; PEREZRUIZ T; MARTINEZLOZANO C;  
HERNANDEZCORDOBA M  
Corporate Source: FAC CIENCIAS MURCIA, CSIC, CTR COORDINADO, DEPT QUIM  
ANAL/MURCIA//SPAIN/  
Journal: ANALES DE QUIMICA, 1977, V73, N1, P67-70  
Language: SPANISH Document Type: ARTICLE  
Geographic Location: SPAIN  
Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth  
Sciences  
Journal Subject Category: CHEMISTRY  
Cited References:  
BARKLEY RA, 1960, V32, P154, ANALYT CHEMISTRY  
BOGNAR J, 1963, V37, P381, ACTA CHIM ACAD SCI H  
CHANEY AL, 1950, V22, P939, ANALYT CHEMISTRY  
CHANEY AL, 1940, V12, P179, IND ENG CHEM ANALYT



DUBRAVCIC M, 1955, V80, P146, ANALYST  
ESPENSON JH, 1964, V86, P5101, J AM CHEM SOC  
FAURE J, 1966, V63, P621, J CHIM PHYS  
JOUSSOTDUBIEN J, 1963, V91, P1214, J CHIM PHYS  
KAZUO S, 1969, P119, TOHUKU U SERV  
LEIN A, 1951, V23, P1507, ANALYT CHEMISTRY  
MASSAE N, 1969, V42, P2464, B CHEM SOC JAPAN  
OSTER G, 1957, V79, P4836, J AM CHEM SOC  
SANDELL EB, 1934, V56, P1426, J AM CHEM SOC  
SIERRA F, 1972, V68, P1091, AN QUIMICA  
SIERRA F, 1974, V70, P595, AN QUIMICA  
SIERRA F, 1975, V78, P498, ANAL CHIM ACTA  
SIERRA F, 1975, V78, P277, ANALYT CHIM  
SIERRA F, 1971, V25, P73, INFORM QUIM ANAL  
SIERRA F, 1973, V27, P93, INFORM QUIM ANAL  
SIERRA F, TO BE PUBLISHED

?t s11/3,kwic/25-48

>>>KWIC option is not available in file(s): 399

11/3,KWIC/25 (Item 1 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

121234606 CA: 121(20)234606w JOURNAL

Use of cetylpyridinium chloride in photogalvanic cell for solar energy  
conversion and storage: thionine-EDTA system

AUTHOR(S): Ameta, Suresh C.; Lodha, Anita; Sahasi, Sapna; Ameta,  
Rameshwar

LOCATION: Univ. Coll. Sci., Sukhadia Univ., Udaipur, 313 001, India

JOURNAL: Proc. Natl. Acad. Sci., India, Sect. A DATE: 1994 VOLUME: 64

NUMBER: 1 PAGES: 43-8 CODEN: PAIAA3 ISSN: 0369-8203 LANGUAGE: English

11/3,KWIC/26 (Item 2 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

118224926 CA: 118(23)224926z JOURNAL

(1,2-Bis(2-hydroxyphenyl)ethylenediamine)dichloroplatinum(II), a new  
compound for the therapy of ovarian cancer. III. Detailed evaluation of the  
antitumor activity of the enantiomeric complexes on the human NIH:OVCAR-3  
ovarian cancer cell line

AUTHOR(S): Bernhardt, Gunther; Gust, Ronald; Reile, Herta; Vom Orde, Hans  
Dieter; Mueller, Richard; Keller, Christoph; Spruss, Thilo; Schoenenberger,  
Helmut; Burgemeister, Thomas; et al.

LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400

JOURNAL: J. Cancer Res. Clin. Oncol. DATE: 1992 VOLUME: 118 NUMBER: 3

PAGES: 209-15 CODEN: JCROD7 ISSN: 0171-5216 LANGUAGE: English

11/3,KWIC/27 (Item 3 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

117082983 CA: 117(9)82983u JOURNAL

Dichloro-(1-(hydroxyphenyl)-2-phenylethylenediamine)platinum(II)  
complexes: testing on the human ovarian cancer cell lines NIH: OVCAR 3  
and SK OV 3

AUTHOR(S): Bernhardt, Guenther; Mueller, Richard; Gust, Ronald; Reile,  
Herta; Keller, Christoph; Spruss, Thilo; Schoenenberger, Helmut

LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400

JOURNAL: Arch. Pharm. (Weinheim, Ger.) DATE: 1992 VOLUME: 325 NUMBER:

2 PAGES: 93-9 CODEN: ARPMAS ISSN: 0365-6233 LANGUAGE: English

11/3,KWIC/28 (Item 4 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**113086948 CA: 113(10)86948b JOURNAL**

**Photogalvanic effect in systems containing basic dyes**

AUTHOR(S): Pezza, Leonardo; Neumann, Miguel Guillermo; Gessner, Fergus

LOCATION: Inst. Biocienc., UNESP, 15100, Sao Jose do Rio Preto, Brazil

JOURNAL: Ecletica Quim. DATE: 1989 VOLUME: 14, PAGES: 27-37 CODEN:

ECQUDX ISSN: 0100-4670 LANGUAGE: Portuguese

**11/3,KWIC/29 (Item 5 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**107069877 CA: 107(8)69877m JOURNAL**

**The reaction between thionine blue and EDTA as a photochemical pretreatment in determinations of chromium(VI) and peroxydisulfate**

AUTHOR(S): Martinez Lozano, C.; Tomas Martinez, V.; Yague, E.

LOCATION: Cent. Coord., CSIC, Spain,

JOURNAL: Afinidad DATE: 1987 VOLUME: 44 NUMBER: 408 PAGES: 115-18

CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

**11/3,KWIC/30 (Item 6 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**106226561 CA: 106(26)226561c JOURNAL**

**Thionine blue as a photoredox indicator in titrations with EDTA.**

**Determinations of metal ions, mixtures, and anions**

AUTHOR(S): Perez Ruiz, T.; Martinez Lozano, C.; Tomas, V.; Yague, E.

LOCATION: Fac. Cienc. Quim. Mat., Univ. Murcia, Murcia, Spain,

JOURNAL: Quim. Anal. (Barcelona) DATE: 1986 VOLUME: 5 NUMBER: 2

PAGES: 164-79 CODEN: QUANEL ISSN: 0212-0569 LANGUAGE: Spanish

**11/3,KWIC/31 (Item 7 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**101113892 CA: 101(14)113892q JOURNAL**

**Thionine and ferric chelate compounds as coupled mediators in microbial fuel cells**

AUTHOR(S): Tanaka, Kazuko; Vega, Carmen A.; Tamamushi, Reita

LOCATION: Inorg. Chem. Lab., Inst. Phys. Chem. Res., Wako, Japan, 351

JOURNAL: Bioelectrochem. Bioenerg. DATE: 1983 VOLUME: 11 NUMBER: 4-6

PAGES: 289-97 CODEN: BEBEBP ISSN: 0302-4598 LANGUAGE: English

**11/3,KWIC/32 (Item 8 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**99178976 CA: 99(22)178976e JOURNAL**

**Photochemical energy conversion by a thiazine photosynthetic-photoelectrochemical cell**

AUTHOR(S): Pan, R. L.; Bhardwaj, R.; Gross, E. L.

LOCATION: Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA

JOURNAL: J. Chem. Technol. Biotechnol., Chem. Technol. DATE: 1983

VOLUME: 33A NUMBER: 1 PAGES: 39-48 CODEN: JCTTDW ISSN: 0142-0356

LANGUAGE: English

**11/3,KWIC/33 (Item 9 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**98071284 CA: 98(9)71284e JOURNAL**  
**Effect of DNA and other polyanions on the EDTA induced photoreduction of thionine**  
AUTHOR(S): Pal, Medini Kanta; Manna, Pravash C.  
LOCATION: Fac. Sci., Univ. Kalyani, Kalyani, 741235, India  
JOURNAL: Makromol. Chem. DATE: 1982 VOLUME: 183 NUMBER: 11 PAGES: 2811-21  
CODEN: MACEAK ISSN: 0025-116X LANGUAGE: English

**11/3,KWIC/34 (Item 10 from file: 399)**  
DIALOG(R) File 399:CA SEARCH(R)  
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**93060423 CA: 93(6)60423v JOURNAL**  
**The use of thionine as a photoredox indicator in amperometric titrations of the mercury(I) ion with polyaminopolycarboxylated agents**  
AUTHOR(S): Sierra, F.; Cebrian, A.; Hidalgo de Cisneros, J. L. H.  
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain,  
JOURNAL: Afinidad DATE: 1979 VOLUME: 36 NUMBER: 364 PAGES: 515-17  
CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

**11/3,KWIC/35 (Item 11 from file: 399)**  
DIALOG(R) File 399:CA SEARCH(R)  
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**88145569 CA: 88(20)145569u JOURNAL**  
**Determination of periodate with photoreduced thionine**  
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.  
LOCATION: Dep. Anal. Chem., Univ. Murcia, Murcia, Spain  
JOURNAL: Anal. Chim. Acta DATE: 1977 VOLUME: 94 NUMBER: 1 PAGES: 129-33  
CODEN: ACACAM ISSN: 0003-2670 LANGUAGE: English

**11/3,KWIC/36 (Item 12 from file: 399)**  
DIALOG(R) File 399:CA SEARCH(R)  
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**88083095 CA: 88(12)83095a JOURNAL**  
**Determination of trace iodide by using its inhibiting effect on the photochemical reaction between thionine and EDTA**  
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.; Hernandez Cordoba, M.  
LOCATION: Dep. Quim. Anal., CSIC, Murcia, Spain  
JOURNAL: An. Quim. DATE: 1977 VOLUME: 73 NUMBER: 1 PAGES: 67-70  
CODEN: ANQUBU LANGUAGE: Spanish

**11/3,KWIC/37 (Item 13 from file: 399)**  
DIALOG(R) File 399:CA SEARCH(R)  
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**86162741 CA: 86(22)162741v JOURNAL**  
**Photoinduced potentials by photoredox systems and the effects of the addition of EDTA**  
AUTHOR(S): Kaneko, Masao; Yamada, Akira  
LOCATION: Inst. Phys. Chem. Res., Wako, Japan  
JOURNAL: Rikagaku Kenkyusho Hokoku DATE: 1976 VOLUME: 52 NUMBER: 6  
PAGES: 210-15 CODEN: RKKHAO LANGUAGE: Japanese

**11/3,KWIC/38 (Item 14 from file: 399)**  
DIALOG(R) File 399:CA SEARCH(R)  
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**85195209 CA: 85(26)195209k TECHNICAL REPORT**

**Evaluation of some thionine redox systems as potential regenerative photogalvanic batteries**

AUTHOR(S): Fine, Dwight A.; Fletcher, Aaron N.  
LOCATION: Nav. Weapons Cent., China Lake, Calif.  
JOURNAL: U. S. NTIS, AD Rep. DATE: 1976 NUMBER: AD-A021424 PAGES: 25 pp. CODEN: XADRCH LANGUAGE: English CITATION: Gov. Rep. Announce. Index (U. S.) 1976, 76(9), 121 AVAIL: NTIS

**11/3,KWIC/39** (Item 15 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

**82154984 CA: 82(23)154984d JOURNAL**  
**Photochemical determination of a new acid-base equilibrium of thionine in its triplet state. Application to the photoreactivity of thiazine dyes in aqueous solution**

AUTHOR(S): Bonneau, Roland; Pereyre, Josette; Jousset-Dubien, Jacques  
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I 351, Talence, Fr.  
JOURNAL: Mol. Photochem. DATE: 1974 VOLUME: 6 NUMBER: 3 PAGES: 245-52  
CODEN: MLPCBL LANGUAGE: English

**11/3,KWIC/40** (Item 16 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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**81019300 CA: 81(4)19300s PATENT**

**Photochromic polymers**

INVENTOR(AUTHOR): Hasegawa, Haruo  
ASSIGNEE: Ricoh Co., Ltd.  
PATENT: Japan Kokai Tokkyo Koho JP 7401690 DATE: 740109  
APPLICATION: Japan JP 7238911 DATE: 720418  
PAGES: 5 pp. CODEN: JKXXAF CLASS: 26(3)F115

**11/3,KWIC/41** (Item 17 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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**79100138 CA: 79(16)100138a JOURNAL**  
**Photochemical redox indicators in amperometry. Indirect determinations of anions with disodium dihydrogen ethylenediaminetetraacetate**

AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.  
LOCATION: Cons. Super. Invest. Cient., Murcia, Spain  
JOURNAL: Inform. Quim. Anal. DATE: 1973 VOLUME: 27 NUMBER: 2 PAGES: 93-8 CODEN: IFQAAZ LANGUAGE: Spanish

**11/3,KWIC/42** (Item 18 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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**79043647 CA: 79(8)43647q JOURNAL**  
**Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis. I**

AUTHOR(S): Bonneau, Roland; Jousset-Dubien, Jacques; Faure, Jean  
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I, Talence, Fr.  
JOURNAL: Photochem. Photobiol. DATE: 1973 VOLUME: 17 NUMBER: 5  
PAGES: 313-19 CODEN: PHCBAP LANGUAGE: English

**11/3,KWIC/43** (Item 19 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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78066493 CA: 78(10)66493e JOURNAL  
Photoreduction indicators in amperometry. Titration of mercury(II),  
zinc(II), and calcium(II) with ethylenediaminetetraacetic acid  
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Martinez Lozano, C.; Perez  
Ruiz, T.  
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain  
JOURNAL: An. Quim. DATE: 1972 VOLUME: 68 NUMBER: 9-10 PAGES: 1091-6  
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/44 (Item 20 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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76080559 CA: 76(14)80559w JOURNAL  
Use of photosensitizers in some volumetric determinations of reduction  
AUTHOR(S): Martinez Lozano, Maria Del C.  
LOCATION: Univ. Murcia, Murcia, Spain  
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1970 VOLUME: 28 NUMBER:  
1-2-3-4 PAGES: 127-97 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/45 (Item 21 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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74008313 CA: 74(2)8313c JOURNAL  
Photochemical reduction of thionine and other thiazine dyes by Co(II)EDTA  
complex in a heterogeneous system  
AUTHOR(S): Singhal, G. S.; Rabinowitch, Eugene  
LOCATION: Chem. Dep., State Univ. New York, Albany, N. Y.  
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 53 NUMBER: 10 PAGES:  
4109-10 CODEN: JCPSA6 LANGUAGE: English

11/3,KWIC/46 (Item 22 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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73051836 CA: 73(10)51836s JOURNAL  
Formation and stabilization of high valence states in silver  
AUTHOR(S): Sanchez-Manzanares, Jose A.  
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1968 VOLUME: 26 NUMBER: 1-4  
PAGES: 57-161 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/47 (Item 23 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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72122159 CA: 72(24)122159r JOURNAL  
Photopolymerization using methylene blue-reducing agent system and its  
application to photoelectrochemical cell  
AUTHOR(S): Kamiya, Nobuyuki; Okawara, Makoto  
LOCATION: Res. Lab. Resour. Util., Tokyo Inst. Technol., Tokyo, Japan  
JOURNAL: Kogyo Kagaku Zasshi DATE: 1969 VOLUME: 72 NUMBER: 12 PAGES:  
2639-44 CODEN: KGKZA7 LANGUAGE: Japanese

11/3,KWIC/48 (Item 24 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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72084849 CA: 72(16)84849f JOURNAL  
Photochemical reduction of thionine by cobalt(II) EDTA complex in  
water-ether emulsion  
AUTHOR(S): Srinivasan, V.; Rabinowitch, E.

LOCATION: Dep. of Bot., Univ. of Illinois, Urbana, Ill.  
JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 52 NUMBER: 3 PAGES: 1165-8  
CODEN: JCPSA6 LANGUAGE: English  
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\$0.95 Estimated cost File155  
\$0.06 0.011 DialUnits File5  
\$2.00 1 Type(s) in Format 9  
\$2.00 1 Types  
\$2.06 Estimated cost File5  
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\$12.86 2 Type(s) in Format 9  
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\$0.05 0.011 DialUnits File35  
\$0.05 Estimated cost File35  
\$0.06 0.011 DialUnits File48  
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\$0.04 0.011 DialUnits File65  
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\$0.09 0.011 DialUnits File71  
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\$0.12 0.011 DialUnits File73  
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\$0.04 0.011 DialUnits File94  
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\$0.03 0.011 DialUnits File98  
\$0.03 Estimated cost File98  
\$0.06 0.011 DialUnits File135  
\$0.06 Estimated cost File135  
\$0.09 0.022 DialUnits File144  
\$8.25 5 Type(s) in Format 9  
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\$8.34 Estimated cost File144  
\$0.05 0.011 DialUnits File149  
\$0.05 Estimated cost File149  
\$0.06 0.011 DialUnits File156  
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\$0.03 0.011 DialUnits File159  
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\$0.05 0.011 DialUnits File162  
\$0.05 Estimated cost File162  
\$0.04 0.011 DialUnits File164  
\$0.04 Estimated cost File164  
\$0.12 0.011 DialUnits File172  
\$0.12 Estimated cost File172  
\$0.04 0.011 DialUnits File266  
\$0.04 Estimated cost File266  
\$0.04 0.011 DialUnits File369  
\$0.04 Estimated cost File369  
\$0.04 0.011 DialUnits File370  
\$0.04 Estimated cost File370  
\$1.40 0.111 DialUnits File399  
\$66.00 24 Type(s) in Format 3  
\$66.00 24 Types  
\$67.40 Estimated cost File399  
\$0.49 0.022 DialUnits File434  
\$6.43 1 Type(s) in Format 9  
\$6.43 1 Types  
\$6.92 Estimated cost File434  
\$0.05 0.011 DialUnits File444  
\$0.05 Estimated cost File444

11/3,KWIC/40 (Item 16 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

81019300 CA: 81(4)19300s PATENT  
Photochromic polymers  
INVENTOR(AUTHOR): Hasegawa, Haruo  
ASSIGNEE: Ricoh Co., Ltd.  
PATENT: Japan Kokai Tokkyo Koho JP 7401690 DATE: 740109  
APPLICATION: Japan JP 7238911 DATE: 720418  
PAGES: 5 pp. CODEN: JKXXAF CLASS: 26(3)F115

11/3,KWIC/41 (Item 17 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

79100138 CA: 79(16)100138a JOURNAL  
Photochemical redox indicators in amperometry. Indirect determinations  
of anions with disodium dihydrogen ethylenediaminetetraacetate  
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez  
Lozano, C.  
LOCATION: Cons. Super. Invest. Cient., Murcia, Spain  
JOURNAL: Inform. Quim. Anal. DATE: 1973 VOLUME: 27 NUMBER: 2 PAGES:  
93-8 CODEN: IFQAAZ LANGUAGE: Spanish

11/3,KWIC/42 (Item 18 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

79043647 CA: 79(8)43647q JOURNAL  
Mechanism of photoreduction of thiazine dyes by EDTA studied by flash  
photolysis. I  
AUTHOR(S): Bonneau, Roland; Joussot-Dubien, Jacques; Faure, Jean  
LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I, Talence, Fr.  
JOURNAL: Photochem. Photobiol. DATE: 1973 VOLUME: 17 NUMBER: 5  
PAGES: 313-19 CODEN: PHCBAP LANGUAGE: English

11/3,KWIC/43 (Item 19 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

78066493 CA: 78(10)66493e JOURNAL  
Photoreduction indicators in amperometry. Titration of mercury(II),  
zinc(II), and calcium(II) with ethylenediaminetetraacetic acid  
AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Martinez Lozano, C.; Perez  
Ruiz, T.  
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain  
JOURNAL: An. Quim. DATE: 1972 VOLUME: 68 NUMBER: 9-10 PAGES: 1091-6  
CODEN: ANQUBU LANGUAGE: Spanish

11/3,KWIC/44 (Item 20 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

76080559 CA: 76(14)80559w JOURNAL  
Use of photosensitizers in some volumetric determinations of reduction  
AUTHOR(S): Martinez Lozano, Maria Del C.  
LOCATION: Univ. Murcia, Murcia, Spain  
JOURNAL: An. Univ. Murcia, Cienc. DATE: 1970 VOLUME: 28 NUMBER:  
1-2-3-4 PAGES: 127-97 CODEN: AUMCB5 LANGUAGE: Spanish

11/3,KWIC/45 (Item 21 from file: 399)

#### Detailed Description

... capable of binding antigen or antibodies. well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified cellulose, polyacrylamide, agarose, and magnetite.

The nature of...metals can be attached to the TNF specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic acid (DTPA)** or **ethylenediaminetetraacetic acid (EDTA)**.

The antibody also can be detectably labeled by coupling to a chemiluminescent compound. The presence...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...plus protease inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 gg/ml; PMSF, 1mM, and **EDTA**, 1mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and centrifuged...then centrifuged at 10,000 G. These supernatants were supplemented

- 42  
with protease inhibitors (PMSF, **EDTA**, EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...12% glycerol, 12mM HEPES (pH 7.9), 4mM Tris (pH 7.9), 60mM KCl, 1mM **EDTA**, and 1mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh.

The ...non-denaturing polyacrylamide gel (270 Al 1M Tris, pH 7.9; 80 til 0.5M **EDTA**, pH 7.9; 13.2 Al 1M sodium acetate, pH 7.9; 5.33 ml...

...7.9; 13.2 ml 1M sodium Acetate, pH 7.9; 8 ml 0.5M **EDTA**, pH 8.0; up to a final volume of 4 liters with ddH<sub>2</sub>O). Gels were...

15/3,KWIC/52 (Item 21 from file: 349)

DIALOG(R)File 349:PCT FULLTEXT

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00300078

#### ANTIMICROBIAL PROTEINS

#### PROTEINES ANTIMICROBIENNES

Patent Applicant/Assignee:

ZENECA LIMITED,  
BROEKAERT Willem Frans,  
CAMMUE Bruno Philippe Angelo,  
OSBORN Rupert William,  
REES Sarah Bronwen,

Inventor(s):

BROEKAERT Willem Frans,  
CAMMUE Bruno Philippe Angelo,  
OSBORN Rupert William,  
REES Sarah Bronwen,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9518229 A1 19950706

Application: WO 94GB2766 19941219 (PCT/WO GB9402766)

Priority Application: GB 9326424 19931224

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AU BB BG BR BY CA CN CZ FI GE HU JP KG KP KR KZ LK LT LV MD MG MN NO NZ  
PL RO RU SI SK TJ TT UA US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR  
IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG



Publication Language: English  
Fulltext Word Count: 7917

Fulltext Availability:  
Detailed Description

#### Detailed Description

... 533-539). Such proteins, including Sia2 from sorghum and SURTMM SHEET (RULE 26) g-l- **purothionin** (g-lP) from wheat, are known to inhibit insect  $\alpha$ -amylase and may be toxic...containing 10 mM NaH<sub>2</sub>PO<sub>4</sub> 15 mM Na<sub>2</sub>HPO<sub>4</sub> 100 mM KCl, 2 mM **EDTA**, 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), it (w/v) SDS, 1 mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, it (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/53 (Item 22 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00293545

COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME

COMPOSITIONS SE FIXANT SPECIFIQUEMENT A DES CELLULES CANCEREUSES COLO-RECTALES ET PROCEDES D'UTILISATION

Patent Applicant/Assignee:

THOMAS JEFFERSON UNIVERSITY,  
WALDMAN Scott A,

Inventor(s):

WALDMAN Scott A,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9511694 A1 19950504

Application: WO 94US12232 19941026 (PCT/WO US9412232)

Priority Application: US 93141892 19931026; US 94305056 19940913

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU JP KE KG KP KR  
KZ LK LR LT LU LV MD MG MN MW NL NO NZ PL PT RO RU SD SE SI SK TJ TT UA  
US US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE  
BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 42481

Fulltext Availability:  
Detailed Description  
Claims

#### Detailed Description

... g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin.

1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 flucrouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 114

CM32 Chromatography

The pullulanase inhibitor sample...30 mM Tris

HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...in wheat flour is about 0.01 % (Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

15/3,KWIC/45 (Item 14 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00476374

**COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME**

**COMPOSITIONS QUI SE LIENT SPECIFIQUEMENT AUX CELLULES CANCEREUSES COLORECTALES ET UTILISATION DE CES COMPOSITIONS**

Patent Applicant/Assignee:

THOMAS JEFFERSON UNIVERSITY,

Inventor(s):

WALDMAN Scott A,  
PEARLMAN Joshua M,  
BARBER Michael T,  
SCHULZ Stephanie,  
PARKINSON Scott J,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9907726 A1 19990218

Application: WO 98US16440 19980807 (PCT/WO US9816440)

Priority Application: US 97908643 19970807

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Publication Language: English

Fulltext Word Count: 36129

Fulltext Availability:

Detailed Description

Detailed Description

... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite.

The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid ( **EDTA** ).

One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... ofmethotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine,

mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene glycol**. The injectable must be sterile and free of pyrogens.

The vaccines of the present...mM sodium deoxycholate, 3.5 mM sodium dodecyl sulfate, 0.5 @tg/mL leupeptin, ImM **EDTA**, 1 gg/mL pepstatin, and 0.2 MM PMSF.

Protein concentrations were determined using the...

15/3,KWIC/46 (Item 15 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00445243

**COMPOSITIONS THAT BIND TO PANCREATIC CANCER CELLS AND METHODS OF USING THE SAME**  
**COMPOSITIONS QUI SE FIXENT SUR LES CELLULES CANCEREUSES PANCREATIQUES ET LEUR MODE D'UTILISATION**

Patent Applicant/Assignee:

THOMAS JEFFERSON UNIVERSITY,  
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WALDMAN Scott A,  
BARBER Michael T,  
BISWAS Sanjoy,

Inventor(s):

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Patent and Priority Information (Country, Number, Date):

Patent: WO 9835707 A1 19980820  
Application: WO 98US3168 19980218 (PCT/WO US9803168)  
Priority Application: US 9738063 19970218

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM  
GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX  
NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH  
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI  
FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 23391

Fulltext Availability:

Detailed Description

Detailed Description

... cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics 5 include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain,

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Patent Applicant/Inventor:

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Patent and Priority Information (Country, Number, Date):

Patent: WO 200162288 A1 20010830 (WO 0162288)  
Application: WO 2001US5897 20010223 (PCT/WO US0105897)  
Priority Application: US 2000185026 20000225

Designated States:

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AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM  
TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 28454

Fulltext Availability:

Detailed Description

Detailed Description

... myeloma lines such as NS-1 or P3NS-1, in the presence of, e.g.,  
**polyethylene glycol.**

The hybridomas or lymphoblastoid cells which secrete antibody of interest can be identified by...doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, n-dtomyacin, bleomycin, **purothionin**, macromomycin, 1,4benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, 5 Clostridium...

15/3,KWIC/36 (Item 5 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00784061

**NUCLEIC ACID SEQUENCES ENCODING CELL WALL-DEGRADING ENZYMES AND USE TO  
ENGINEER RESISTANCE TO FUSARIUM AND OTHER PATHOGENS  
SEQUENCES D'ACIDE NUCLEIQUE CODANT POUR DES ENZYMES DEGRADANT LES PAROIS  
CELLULAIRES ET LEUR UTILISATION POUR CREER UNE RESISTANCE AU FUSARIUM  
ET A D'AUTRES PATHOGENES**

Patent Applicant/Assignee:

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Legal Representative:

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Patent and Priority Information (Country, Number, Date):

Patent: WO 200116353 A1 20010308 (WO 0116353)

Application: WO 2000US23802 20000830 (PCT/WO US0023802)

Priority Application: US 99151582 19990830; US 2000224946 20000811; US 2000649747 20000828

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AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CZ DE DK DM DZ EE ES  
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU  
LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR  
TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 40894

Fulltext Availability:

Detailed Description

Detailed Description

... leaves were inhibitory to *F. solani* (Molina et al., 1993). A combination of a wheat **purothionin** and a 2S albumin from radish or oilseed rape was effective against the growth of...in 0.9 M NaCl, 0.09

M Tris-HCl pH 7.6, 6 mM **EDTA**, 0.5% NP-401 IX Denhardt's solution, 1 mM sodium pyrophosphate, 1 mM sodium...were partitioned on 1W agarose in 40 mM TRIS

acetate, pH 8.21 1 mM **EDTA**, stained with an ethidium bromide solution (0.1 pg/mL), and visualized by irradiation with an...containing 0.5 M

sucrose, 80 mM potassium chloride, 10 mM TRIS-chloride, 10 mM

**EDTA**, 4 mM spermine, 1 mM spermidine, pH 9.51 180 mg/L

phenylmethylsulfonyl fluoride (added ...ML

of a buffer solution containing 50 mM TRIS-chloride, pH 8.1 100 mM

- 95

**EDTA**, 100 mM sodium chloride, and 600  $\mu$ g of proteinase K. An additional 4 mL of...

...extraction buffer containing 50 mM TRIS-chloride, pH 8.1 100 mM sodium chloride, 10 mM **EDTA**, pH 8.1 10W sodium dodecyl sulfate, and 10 mM beta-mercaptoethanol (added immediately before use...extraction buffer (10 mM sodium chloride, 10 mM TRIS-Cl, pH 9.0j, 1 mM **EDTA**), 2.5 mL phenol (saturated with TRIS buffer at pH 4.31 Fisher #BP1751), and...

...formaldehyde (Fisher #BP531), 20 mM MOPS, pH 7.01 5 mM sodium acetate, 10 mM **EDTA**, and 0.01% bromophenol blue. RNA samples were heated at 60°C for 10 to 15...

...was

dissolved in 30 mL of 20 mM MOPS, 5 mM sodium acetate, 10 mM **EDTA**, pH 7.0, to which was added 37 % formaldehyde for a final concentration of 6...

...M sodium phosphate buffer, pH

7.01 0.25 M sodium chloride, and 1 mM **EDTA**, pH 8.0, as recommended by Bio-Rad. Probes for northern blots consisted of partial...consisting of 89 mM TRIS-Cl, pH 8.31 89 mM borate, 1.5 mM

**EDTA**. The resulting endochitinase cleavage products, shown in FIG. 16, closely matched those expected from the...

activity against *Fusarium* and *Trichoderma* were inactive against *Aspergillus flavus*, *Phytophthora parasitica* and other pathogens (Yun et al. (1996)). The differential activities of the chitinases are attributed to inherent properties of the enzymes (Sela-Buurlage et al. 1993, Brunner et al. 1998), to differences in cell wall architecture (Sivan and Chet 1989a, Van Loon 1997) among the fungi, or to other factors.

#### Other Anti-Fusarium Proteins.

Additional types of proteins have been found to have anti-*Fusarium* activity *in vitro* or *in planta*. Boyapati et al. (1994) reported a cysteine protease inhibitor from pearl millet that inhibited the growth of *Fusarium moniliforme* in culture. A cysteine-rich polypeptide from *Impatiens balsamina* seeds was active against *F. culmorum* (Tailor et al. 1997). Cecropin A, a polypeptide from the *Cecropia* moth, was a potent inhibitor of both *F. moniliforme* and *F. oxysporum* (deLucca et al. 1997, Cavallarin et al. 1998). Antifungal proteins from seeds of sorghum had activity against *F. moniliforme* (Seetharaman et al. 1997), and two wheat seed proteins of the PR4 family of pathogenesis-related proteins inhibited hyphal growth of *F. culmorum* and *F. graminearum* (Caruso et al. 1996). Hu and Reddy (1997) isolated a thaumatin-like protein from *Arabidopsis thaliana* with activity against *F. oxysporum*. Non-specific lipid transfer proteins from barley and maize leaves were inhibitory to *F. solani* (Molina et al., 1993). A combination of a wheat purothionin and a 2S albumin from radish or oilseed rape was effective against the growth of *F. culmorum* *in vitro* (Terras et al. 1993).

#### Microbial Genes as Anti-Fungal Transgenes in Plants.

A majority of antifungal genes that have been examined both *in vitro* and *in planta* are of plant origin. To our knowledge, there are two examples of genes from fungi that exhibit antifungal activity. Endochitinases from the parasitic fungus *Trichoderma harzianum* conferred activity against *Alternaria alternata* and *B.*

In accordance with this discovery, it is an object of the invention to provide nucleic acid sequences encoding fungal cell wall-degrading enzymes selected from the group consisting of glucanase, exochitinase, and endochitinase; isolated polypeptides having glucanase, endochitinase or exochitinase activity; recombinant nucleic acid molecules including expression vectors encoding polypeptides having cell wall-degrading activity; and cells harboring the recombinant nucleic acid molecules or expression vectors.

It is also an object of the invention to provide transformation vectors comprising a cell wall-degrading recombinant molecule, which vectors are effective for stably introducing the recombinant molecule into a plant.

It is also an object of the invention to provide methods of producing and using polypeptides having glucanase, endochitinase or exochitinase activity.

It is another object of the invention to provide transgenic plants having bacterial or fungal resistance, wherein the resistance is a result of expression of a recombinant nucleic acid molecule of the invention.

A further object of the invention is to provide fungal genes which generate cell wall-degrading enzymes, including proteins having the capability of degrading the glucan and chitin cell wall components of *F. venenatum* and other *Fusarium* species, including *F. graminearum* and *F. culmorum*, the principle causal agents of head blight (scab) in the U.S.

Another object of the invention is expression of the cell wall-degrading enzymes in transgenic monocots, including wheat, barley or oats, to confer partial or complete resistance to *Fusarium* species and/or to other fungal pathogens of wheat and other cereal crops. Such transgenic lines will be useful genetic stocks for generating improved crops.

A still further object of the invention is the provision of novel wheat germplasms that express genes designed to limit the spread of the pathogenic fungus *Fusarium* and indirectly to curtail the accumulation of DON in infected heads.

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LOZANO Rosa M, Olivos de Penanevada, 5-8degreesA, E-28937 Mostoles, ES,  
WONG Joshua H, 9 Viewmont Terrace, South San Francisco, CA 94080, US,  
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FRICK Oscar L, 370 Parnassus Avenue, San Francisco, CA 94117, US,  
Legal Representative:  
SMITH Karen S (et al) (agent), Flehr Hohbach Test Albritton & Herbert  
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Patent and Priority Information (Country, Number, Date):

Patent: WO 200044781 A1 20000803 (WO 0044781)  
Application: WO 2000US1958 20000125 (PCT/WO US0001958)  
Priority Application: US 99238379 19990127

Designated States:

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AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB  
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG  
UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 44560

Fulltext Availability:

Detailed Description

Detailed Description

... are soluble cereal seed proteins, rich  
in cystine. In the Johnson, et al. investigation, wheat **purothionin** was  
experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR)  
and thioredoxin h according to Eqs. 2 and 3.

(2) NADPH + Thioredoxin h @-@NADP +  
M

Thioredoxin h,ed

(3) **Purothionin** , , , , + Thioredoxin **Purothionin** , ,  
,d  
+ Thioredoxin ho,,

Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice  
...measures are taken to minimize shock, renal failure and respiratory  
failure. Other than administering calcium- **EDTA** in the vicinity of the  
bite  
and excising the wound area, there are no known...PAGE (Coomassie Blue  
stain), but in certain preparations, the band was not sharp.

Other proteins

**Purothionin** a from bread wheat and **purothionins** ; a-1 and 0 from  
durum

wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones,  
respectively. The **purothionin** a sample contained two members of the  
**purothionin** family when examined with SDS-polyacrylamide gel  
electrophoresis. The **purothionin** a-1 and 0 samples were both  
homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried

out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 MM  
**EDTA** and 16 % glycerol in a final volume of 0.1 ml. As indicated, 0.7  
...

...extraplastidic proteins, this test has proved useful in several studies.



A case in point is **purothionin** which, when reduced by thioredoxin h  
activates chloroplast FBPase (Wada, K. et al. (1981).

...and DSG-2) were found to  
be effective in enzyme activation; however, they differed from  
**purothionin** in showing a specificity for NADP-MDH rather than FBPase  
(Table I).

The  $\alpha$ -amylase...2 0  
Ovoinhibitor 49 14 1 0  
Bovine lung (Aprotinin) 7 3 Trace 2  
Thionins  
" **Purothionin** -cil 6 4 1 39  
\*\* **Purothionin** -0 6 4 Trace 5  
tPurothionin-a 6 4 0 14  
These values compare to...

...same as  
for the DSG/DTNB assay except that the DSG proteins were omitted and  
**purothionin** a, 20 ttg or CM-1, 20 ttg was used). The results thus  
confirmed  
the...fluorescent band migrating behind thioredoxin.

#### EXAMPLE 5

Thioredoxin-linked Reduction of

Other Trypsin Inhibitors and **Purothionins**

In view of the finding that cystine-rich trypsin inhibitors from seeds  
can undergo specific...

...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin-reduced **purothionin**  
consistently activated FBPase and the type tested earlier, **purothionin**  
-a,  
failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS  
Lett. 124:237-240). However, in contrast to **purothionin** -a from bread  
wheat, two **purothionins** previously not examined (**purothionins** a-1 and  
from durum wheat) detectably activated NADP-MDH (Table I). The two  
durum wheat **Purothionins** also differed in their ability to activate  
FBPase.

The activity differences between these **purothionins** were unexpected in  
view of the strong similarity in their amino acid sequences (Jones, B...

...to undergo reduction  
by thioredoxin. A requirement for thioredoxin was observed for the  
reduction of **purothionin** (here the a-type) by the SDS-PAGE fluorescence  
procedure.

#### EXAMPLE 6

Quantitation of Reduction...Procedure

The following concentrations of proteins were used (nmoles): thioredoxin,  
0.08; NTR, 0.01; **purothionin** -0, 1.7; DSG-1, 0.7; corn kernel trypsin  
inhibitor, 1.0; Bowman-Birk...

...difference, other conditions

were as in Examples 1

% Reduction After

Protein 20 min 120 min

**Purothionin** -0 15 32

DSG-1 22 38

Corn kernel trypsin inhibitor 3 15

Bowman-Birk...

...CM-1  $\alpha$ -amylase inhibitors (147  
and 210%, respectively); corn kernel trypsin inhibitor (424%); and  
**purothionin** (82, 133, and 120% for the a, al and 0 forms,  
respectively).

Glutaredoxin was ineffective...

...and ovomucoid inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins**, two  $\alpha$ -amylase inhibitors (DSG-1, CM-1), a cysteine-rich trypsin inhibitor from plants... 0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 30°C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0 and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor... HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

#### CM32 Chromatography

The pullulanase inhibitor sample...

... 30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**. Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against... in wheat flour is about 0.01 % (Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

... mM Tris-HCl pH 7.4 containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM **EDTA** -Na] and stirred gently for 30 min at room temperature. The mixture was then centrifuged...

15/3, KWIC/41 (Item 10 from file: 349)

DIALOG(R) File 349: PCT FULLTEXT

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00577322

#### SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

#### FRAGMENTS D'ADN DETERMINES SELON LEUR SEQUENCE ET POLYPEPTIDES CORRESPONDANTS CODES PAR LESDITS FRAGMENTS

Patent Applicant/Assignee:

CERES INC, 3007 Malibu Canyon Road, Malibu, CA 90265, US, US (Residence), US (Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

ALEXANDROV Nikolai, 1404 Oak Trail Street, Thousand Oaks, CA 91320, US, US (Residence), RU (Nationality), (Designated only for: US)

BROVER Vyacheslav, 5916 N. Las Virgenes Road #590, Calabasas, CA 91320, US, US (Residence), RU (Nationality), (Designated only for: US)

CHEN Xianfeng, 12333 Wild Turkey Court, #B, Creve Coeur, MO 63141, US, US (Residence), CN (Nationality), (Designated only for: US)

SUBRAMANIAN Gopalakrishnan, 4205 Peach Slope Road, Moorpark, CA 93021, US, US (Residence), IN (Nationality), (Designated only for: US)

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ZHENG Liansheng, 19212 Circle Gate Drive #201, Germantown, MD 20874, US, US (Residence), CN (Nationality), (Designated only for: US)

Legal Representative:

STEWART Raymond C (et al) (agent), Birch, Stewart, Kolasch & Birch, LLP, P.O. Box 747, Falls Church, VA 22040-0747, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200040695 A2-A3 20000713 (WO 0040695)

Application: WO 2000US466 20000107 (PCT/WO US0000466)

Priority Application: US 99115293 19990108

Designated States:

(Protection type is "patent" unless otherwise stated - for applications

Summary of the Invention:

...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:

...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin**, and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA**, 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed ...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA**. The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in ...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin**, and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin**, an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/18 (Item 17 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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3992551

Derwent Accession: 1996-188141

Utility

C/ Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocorticoid receptor type II and viral protein R interacting protein ; DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I PROTEIN

Inventor: Weiner, David B., Merion, PA

Refaeli, Yosef, Boston, MA

Assignee: The Trustees of the University of Pennsylvania(02), Philadelphia, PA

Pennsylvania, University of (Code: 64664)

Examiner: Adams, Donald E. (Art Unit: 183)

Assistant Examiner: Parkin, Jeffrey S.

Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 5763190            | A    | 19980609 | US 94309644           | 19940921       |

#### Summary of the Invention:

...capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified cellulose, polyacrylamide, agarose, and magnetite. The nature of the...metals can be attached to the TNF-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediaminetetraacetic** acid ( **EDTA** ).

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

#### Description of the Invention:

...inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 [mu]g/ml; PMSF, 1 mM, and **EDTA**, 1 mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and...were then centrifuged at 10,000 G. These supernatants were supplemented with protease inhibitors (PMSF, **EDTA**, EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...HEPES (pH 7.9), 4 mM Tris (pH 7.9), 60 mM KCl, 1 mM **EDTA**, and 1 mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh...polyacrylamide gel (270 [mu]l 1M Tris, pH 7.9; 80 [mu]l 0.5M **EDTA**, pH 7.9; 13.2 [mu]l 1M sodium acetate, pH 7.9; 5.33...

...7.9; 13.2 ml 1M sodium Acetate, pH 7.9; 8 ml 0.5M **EDTA**, pH 8.0; up to a final volume of 4 liters with ddH2O). Gels were...

15/3,KWIC/19 (Item 18 from file: 654)

DIALOG(R)File 654:US Pat.Full.

(c) Format only 2005 The Dialog Corp. All rts. reserv.

3979152

Derwent Accession: 1995-246394

#### Utility

C/ Antimicrobial proteins

; PLANT EXTRACTS

Inventor: Broekaert, Willem Frans, Dilbeek, BE  
Cammue, Bruno Philippe Angelo, Alsemberg, BE  
Osborn, Rupert William, Twickenham, GB  
Rees, Sarah Bronwen, Forest Park, GB

Assignee: Zeneca Agrochemicals(03), GB, England  
Zeneca Agrochemicals GB (Code: 45474)

Examiner: Lebuyader, John (Art Unit: 189)

Assistant Examiner: Wang, Andrew

Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro LLP

|             | Publication<br>Number | Kind | Date          | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|---------------|-----------------------|----------------|
| Main Patent | US 5750504            | A    | 19980512      | US 96656318           | 19960612       |
| PCT         | WO 9518229            |      | 19950706      | WO 94GB2766           | 19941219       |
|             |                       |      | 371:19960612  |                       |                |
|             |                       |      | 102e:19960612 |                       |                |

Fulltext Word Count: 5652

#### Summary of the Invention:

...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

#### Description of the Invention:

15/3,KWIC/23 (Item 22 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2005 The Dialog Corp. All rts. reserv.

3811293

Derwent Accession: 1994-183512

**Utility**

C/ Biocidal proteins

; **BACTERICIDES, FUNGICIDES**

Inventor: Broekaert, Willem F., Dilbeek, BE  
Cammue, Bruno P. A., Alsemberg, BE  
Rees, Sarah B., Forest Park, GB England  
Vanderleyden, Jozef, Heverlee, BE

Assignee: Zeneca Limited(03), London, GB, England  
Zeneca Ltd GB (Code: 32757)

Examiner: Hendricks, Keith D. (Art Unit: 184)

Law Firm: Cushman Darby & Cushman, L.L.P.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 5597801            | A    | 19970128 | US 95451568           | 19950526       |
| Division    | Pending               |      |          | US 93149839           | 19931110       |
| CIP         | Abandoned             |      |          | US 932842             | 19930114       |
| Priority    |                       |      |          | GB 9112300            | 19910607       |
|             |                       |      |          | GB 9223708            | 19921112       |
|             |                       |      |          | GB 933564             | 19930223       |

Fulltext Word Count: 14080

**Description of the Invention:**

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/24 (Item 23 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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3746704

Derwent Accession: 1993-100978

**Utility**

CM/ Biocidal proteins

; **ANTIFUNGAL OR ANTIBACTERIAL AGENTS**

Inventor: Broekaert, Willem F., Dilbeek, BE  
Cammue, Bruno P. A., Alsemberg, BE  
Osborn, Rupert W., Middlesex, GB England  
Rees, Sarah B., Berkshire, GB England  
Terras, Franky R. G., Amzegem, BE  
Vanderleyden, Jozef, Heverlee, BE

Assignee: Zeneca Limited(03), London, GB, England  
Zeneca Ltd GB (Code: 32757)

Examiner: Fox, David T. (Art Unit: 183)

supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine-tetraacetic acid (EDTA)**. One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene glycol**. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):

...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/4 (Item 3 from file: 654)

DIALOG(R) File 654:US Pat.Full.

(c) Format only 2005 The Dialog Corp. All rts. reserv.

0004903757 \*\*IMAGE Available

Derwent Accession: 1993-100978

**Biocidal proteins**

Inventor: Willem Broekaert, INV

Bruno Cammue, INV

Rupert Osborn, INV

Sarah Rees, INV

Franky Terras, INV

Jozef Vanderleyden, INV

Assignee: ZENECA Limited(03)

Correspondence Address: SYNGENTA CROP PROTECTION, INC., 2 RIGHTER PARKWAY P.O. BOX 15458, WILMINGTON, DE, 19850-5458, US

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 20010014732        | A1   | 20010816 | US 2001759584         | 20010112       |
| Division     | US 5538525            |      |          | US 95377687           | 19950125       |
| Continuation | US 6187904            |      |          | US 97971982           | 19971117       |
| Continuation | US 5689043            |      |          | US 95452078           | 19950526       |
| Continuation | ABANDONED             |      |          | US 932480             | 19930104       |
| Continuation | UNKNOWN               |      |          | WO 92GB1570           | 19920827       |
| Priority     |                       |      |          | GB 9118523            | 19910829       |
|              |                       |      |          | GB 923038             | 19920213       |
|              |                       |      |          | GB 9213526            | 19920625       |

Fulltext Word Count: 14428

Summary of the Invention:

...Such proteins, including SI[small alpha, Greek]2 from sorghum and

[small gamma, Greek]-1- **purothionin** from wheat, are known to inhibit insect [small alpha, Greek]-amylase and are toxic to...

Description of the Drawings:

...Lc-AFP, Ct-AMP1, sorghum SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin**, and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:

...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA**, 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA**. The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [small gamma, Greek]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...small alpha, Greek]-amylase inhibitor SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin**, and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...For the purpose of comparison, these tests were performed in parallel with [small beta, Greek]- **purothionin**, an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...with both test fungi. In comparison, the IC[sub]50 value of [small beta, Greek]- **purothionin**

(...Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [small beta, Greek]- **purothionin** administered at 50 [small mu, Greek]g/ml decreased the viability of both cell types...

Exemplary or Independent Claim(s):

...or bacteria which comprises exposure to SI[small alpha, Greek]2, [small gamma, Greek]-1- **purothionin**, or another [small alpha, Greek]-amylase inhibitor protein.

15/3,KWIC/5 (Item 4 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4547046 .

Derwent Accession: 1995-178646

Utility

CERTIFICATE OF CORRECTION

C/ Imaging of colorectal cancer using ST receptor binding compounds ; VISUALIZING TUMOR CELLS; ADMINISTER RADIOACTIVE PARTICLES TO HUMANS AND DETECT POSITIONING AND ACCUMULATION OF RADIOACTIVE PARTICLES IN BODY

Inventor: Waldman, Scott A., Ardmore, PA

Assignee: Thomas Jefferson University(02), Philadelphia, PA  
Jefferson, Thomas University (Code: 06943)

Examiner: Celsa, Bennett (Art Unit: 168)

Assistant Examiner: Ricigliano, Joseph W.

Law Firm: Woodcock Washburn Kurt Mackiewicz & Norris LLP

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6268159            | A    | 20010731 | US 98138237           | 19980821       |
| Division    | Pending               |      |          | US 95468449           | 19950606       |
| Division    | US 5518888            | A    |          | US 93141892           | 19931026       |

Fulltext Word Count: 28331

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

#### Description of the Invention:

...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2... at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA**. Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepentaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/6 (Item 5 from file: 654)  
 DIALOG(R)File 654:US Pat.Full.  
 (c) Format only 2005 The Dialog Corp. All rts. reserv.

4458415 \*\*IMAGE Available  
 Derwent Accession: 1993-100978

#### Utility

##### C/ Biocidal proteins

##### ; AMINO ACID SEQUENCES OF MICROBIOCIDAL PROTEINS; FOR TRANSGENIC PLANTS WITH INCREASED DISEASE RESISTANCE

Inventor: Broekaert, Willem F., Dilbeek, BE  
 Cammue, Bruno P. A., Alseberg, BE  
 Osborn, Rupert W., Middlesex, GB  
 Rees, Sarah B., Berkshire, GB  
 Terras, Franky R. G., Amzegem, BE  
 Vanderleyden, Jozef, Heverlee, BE

Assignee: ZENECA Limited(03), London, GB  
 Zeneca Ltd GB (Code: 32757)

Examiner: Low, Christopher S. F. (Art Unit: 163)

Assistant Examiner: Gupta, Anish

Combined Principal Attorneys: Hohenschutz, Liza D.

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 6187904            | A    | 20010213 | US 97971982           | 19971117       |
| Division     | US 5538525            | A    |          | US 95377687           | 19950125       |
| Continuation | US 5689043            | A    |          | US 95452078           | 19950526       |
| Continuation | Abandoned             |      |          | US 932480             | 19930104       |
| Continuation | Abandoned             |      |          | WO 92GB1570           | 19920827       |
| Priority     |                       |      |          | GB 9118523            | 19910829       |
|              |                       |      |          | GB 923038             | 19920213       |
|              |                       |      |          | GB 9213526            | 19920625       |

Fulltext Word Count: 11346

Summary of the Invention:



...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:

...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin**, and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA**, 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA**. The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored ...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]-**purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin**, and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]-**purothionin**, an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]-**purothionin** (...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml

15/3,KWIC/7 (Item 6 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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4458048

Derwent Accession: 1998-456873

Utility

CERTIFICATE OF CORRECTION

C/ Methods of identifying and detecting pancreatic cancer  
; IN VITRO DIAGNOSIS OF TUMORS IN HUMANS; ANALYZING PREFERENTIAL ORGAN  
TISSUE FOR PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS,  
PRESENCE OF CHOLECYSTOKININ MESSENGER RIBONUCLEIC ACIDS INDICATES HUMAN HAS  
A TUMOR

Inventor: Weinberg, David, Philadelphia, PA

Waldman, Scott A., Ardmore, PA

Barber, Michael T., Paoli, PA

Biswas, Sanjoy, Philadelphia, PA

Assignee: Thomas Jefferson University(02), Philadelphia, PA

Jefferson, Thomas University (Code: 06943)

Examiner: Eyler, Yvonne (Art Unit: 162)

Assistant Examiner: Holleran, Anne L.

Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6187536            | A    | 20010213 | US 9825534            | 19980218       |
| Provisional |                       |      |          | US 60-38063           | 19970218       |

Fulltext Word Count: 23168

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic acid** (DTPA) or **ethylenediamine**-tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as ...

15/3,KWIC/8 (Item 7 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4427576

Derwent Accession: 2000-687452

#### Utility

C/ X-ray guided drug delivery

; TARGETING A TISSUE; EXPOSURE OF TISSUE TO IONIZING RADIATION

Inventor: Hallahan, Dennis E., Nashville, TN

Assignee: Vanderbilt University(02), Nashville, TN

Vanderbilt University (Code: 88418)

Examiner: Schwartzman, Robert A. (Art Unit: 166)

Assistant Examiner: Sandals, William

Law Firm: Jenkins & Wilson, P.A.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 6159443            | A    | 20001212 | US 99302456           | 19990429       |

Fulltext Word Count: 26146

#### Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...

#### Description of the Invention:

...by staining for factor VIII. Confluent cells were harvested with 0.1% collagenase 0.01% **EDTA** and subcultured at a ratio of 1:3. HUVECs were used at third passage;

#### Non-exemplary or Dependent Claim(s):

...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...daunorubicin, cytosine arabinoside, etoposide, 5-4

4317418

Derwent Accession: 1995-178646

**Utility**

**C/ Compositions that specifically bind to colorectal cancer cells and methods of using the same**

**; CONJUGATED COMPOUNDS WHICH COMPRISE AN ST RECEPTOR BINDING MOIETY AND A RADIOSTABLE ACTIVE MOIETY**

Inventor: Waldman, Scott A., Ardmore, PA

Assignee: Thomas Jefferson University(02), Philadelphia, PA  
Jefferson, Thomas University (Code: 06943)

Examiner: Brusca, John S. (Art Unit: 165)

Assistant Examiner: Larson, Thomas G.

Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

|             | Publication<br>Number | Kind | Date          | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|---------------|-----------------------|----------------|
| Main Patent | US 6060037            | A    | 20000509      | US 96635930           | 19960426       |
| CIP         | US 5518888            | A    |               | US 93141892           | 19931026       |
| PCT         | WO 9511694            |      | 19950504      | WO 94US12232          | 19941026       |
|             |                       |      | 371:19960426  |                       |                |
|             |                       |      | 102e:19960426 |                       |                |
|             | US 5601990            | A    |               | US 94305056           | 19940913       |

Fulltext Word Count: 39301

**Description of the Invention:**

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1, ... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin**, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene**, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine-tetraacetic** acid ( **EDTA** ). One skilled in the art would readily recognize other fluorescence-emitting metals as well asCompound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** ( **ethylenediaminetetraacetic** acid) or DTPA ( **diethylenetriaminepetaacetic** acid) . DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/11 (Item 10 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4239629

Derwent Accession: 1994-249225

**Utility**

**REISSUE REQUESTED** \*\*See File 123 for details

**C/ High lysine derivatives of [alpha]-hordothionin**

**; FOR TRANSFORMED PLANTS AND CELLS WITH ENHANCED LYSINE CONTENT AND WHICH SUPPRESS AND KILL PLANT PATHOGENS INCLUDING FUNGI**

Inventor: Rao, A. Gururaj, Urbandale, IA

Beach, Larry, Des Moines, IA

Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA

Pioneer Hi-Bred International Inc (Code: 17947)

Examiner: Smith, Lynette R. F. (Art Unit: 169)

Assistant Examiner: Kimball, Melissa L.

Law Firm: Pioneer Hi-Bred International, Inc.

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 5990389            | A    | 19991123 | US 97838763           | 19970410       |
| Continuation | Abandoned             |      |          | US 95575654           | 19951220       |
| Continuation | Abandoned             |      |          | US 95369975           | 19950106       |
| Continuation | Abandoned             |      |          | US 933885             | 19930113       |

Fulltext Word Count: 4619

**Description of the Invention:**

...crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

**15/3,KWIC/12 (Item 11 from file: 654)**

DIALOG(R)File 654:US Pat.Full.

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4235015

Derwent Accession: 1994-183512

**Utility**

**C/ Transgenic plants expressing biocidal proteins**

**; ISOLATED ANTIMICROBIAL PROTEIN HAVING AMINO ACID SEQUENCECONTAINING COMMON CYSTEINE/GLYCINE DOMAIN OF CHITIN BINDINGPLANT PROTEINS; AGICULTURAL AND PHARMACEUTICAL APPLICATIONS AS FUNGICIDES OR BACETRICIDES**

Inventor: Broekaert, Willem Frans, Dilbeek, BE

Cammue, Bruno Phillippe Angelo, Alseberg, BE

Rees, Sarah Bronwen, Forest Park, GB

Vanderleyden, Jozef, Heverlee, BE

Assignee: Zeneca Limited(03), London, GB

Zeneca Ltd GB (Code: 32757)

Examiner: Kemmerer, Elizabeth (Art Unit: 166)

Combined Principal Attorneys: Hohenschutz, Liza D.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent | US 5986176            | A    | 19991116 | US 96777113           | 19961230       |
| Division    | US 5691199            | A    |          | US 95451566           | 19950526       |
| Division    | US 5514779            | A    |          | US 93149839           | 19931110       |
| CIP         | Abandoned             |      |          | US 932842             | 19930114       |
| CIP         | Pending               |      |          | WO 92GB999            | 19920603       |
| Priority    |                       |      |          | GB 9112300            | 19910607       |
|             |                       |      |          | GB 9223798            | 19921112       |
|             |                       |      |          | GB 933564             | 19930223       |

Fulltext Word Count: 13574

Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...  
sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/13 (Item 12 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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4187951

Derwent Accession: 1992-331736

Utility

C/ Biocidal proteins

; **TRANSFORMED BIOLOGICAL PROTEIN**

Inventor: De Bolle, Miguel, Leuven, BE

Broekaert, Willem Frans, Dilbeek, BE

Cammue, Bruno Philippe Angelo, Alsemberg, BE

Rees, Sarah Bronwen, Bracknell, GB

Vanderleyden, Jozef, Heverlee, BE

Assignee: Zeneca Limited(03), London, GB

Zeneca Ltd GB (Code: 32757)

Examiner: Fox, David T. (Art Unit: 169)

Combined Principal Attorneys: Hohenschutz, Liza D.

|              | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|--------------|-----------------------|------|----------|-----------------------|----------------|
| Main Patent  | US 5942663            | A    | 19990824 | US 97915142           | 19970820       |
| Division     | US 5482928            | A    |          | US 93117080           | 19931220       |
| Continuation | US 5689048            | A    |          | US 95471329           | 19950602       |
| Priority     |                       |      |          | GB 915052             | 19910311       |
|              |                       |      |          | GB 915684             | 19910319       |
|              |                       |      |          | WO 92GB423            | 19920310       |

Fulltext Word Count: 6005

Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The ...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...  
...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and

from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as... time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. Also, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (.circle-solid...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/14 (Item 13 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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4163914

Derwent Accession: 1995-246394

#### Utility

C/ Transformed plants expressing antimicrobial proteins

; ANTIMICROBIAL PROTEIN CAPABLE OF ISOLATION FROM SEEDS OF HEUCHERA OR AESCULUS

Inventor: Broekaert, Willem Frans, Dilbeek, BE  
Cammue, Bruno Philippe Angelo, Alsemberg, BE  
Osborn, Rupert William, Middlesex, GB  
Rees, Sarah Bronwen, Bracknell, GB

Assignee: Zeneca Limited(03), London, GB  
Zeneca Ltd GB (Code: 32757)

Examiner: Robinson, Douglas W. (Art Unit: 169)

Assistant Examiner: Zaghmout, Ousama M-Faiz

Combined Principal Attorneys: Hohenschutz, Liza D.

|             | Publication<br>Number | Kind | Date     | Application<br>Number | Filing<br>Date |
|-------------|-----------------------|------|----------|-----------------------|----------------|
|             | -----                 | --   | -----    | -----                 | -----          |
| Main Patent | US 5919918            | A    | 19990706 | US 97956459           | 19971023       |
| Division    | Pending               |      |          | US 656318             |                |
| Priority    |                       |      |          | GB 9326424            | 19931224       |

Fulltext Word Count: 5637

#### Summary of the Invention:

...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

#### Description of the Invention:

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/15 (Item 14 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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QUICKENDEN TI, 1981, V85, P2232, J PHYS CHEM-US  
 QUICKENDEN TI, 1975, V17, P147, SOL ENERGY  
 QUICKENDEN TI, 1977, V19, P283, SOL ENERGY  
 RABINOWITCH E, 1940, V8, P551, J CHEM PHYS  
 RABINOWITCH E, 1940, V8, P560, J CHEM PHYS  
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 SHIGEHARA K, 1977, V81, P1883, J PHYS CHEM-US  
 SUDA Y, 1978, V82, P268, J PHYS CHEM-US  
 TIEN HT, 1978, V125, P885, J ELECTROCHEM SOC  
 TRAMLER G, 1915, V90, P385, Z PHYS CHEM  
 WILDES PD, 1975, V97, P2288, J AM CHEM SOC  
 WILDES PD, 1978, V100, P6568, J AM CHEM SOC  
 WILDES PD, 1978, V82, P981, J PHYS CHEM-US  
 WILDES PD, 1977, V19, P567, SOL ENERGY  
 WILDES PD, 1978, V19, P597, SOL ENERGY  
 WRIGHTON MS, 1979, V12, P303, ACCOUNTS CHEM RES

11/9/7 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
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02513289 Genuine Article#: LG689 Number of References: 0  
 (NO REFS KEYED)

**Title: USE OF TRITON X-100 IN A PHOTOGALVANIC CELL FOR SOLAR-ENERGY  
 CONVERSION AND STORAGE - THIONINE EDTA SYSTEM**

Author(s): AMETA SC; CHITTORA AK; KHAMESRA S; AMETA R

Corporate Source: SUKHADIA UNIV, UNIV COLL SCI, DEPT CHEM, 15 RADHEYSHYAM  
 ST, BHRAMPOLE GATE/UDAIPUR 313001//INDIA/

Journal: ARABIAN JOURNAL FOR SCIENCE AND ENGINEERING, 1992, V17, N4A (OCT)  
 , P477-480

ISSN: 0377-9211

Language: ENGLISH Document Type: ARTICLE

Geographic Location: INDIA

Subfile: SciSearch; CC ENGI--Current Contents, Engineering, Technology &  
 Applied Sciences

Journal Subject Category: MULTIDISCIPLINARY SCIENCES

Abstract: **Thionine** , **ethylenediaminetetraacetic** acid, and Triton X-100  
 (Tx) have been used as photosensitizer, reductant, and surfactant,  
 respectively in a photogalvanic cell for solar energy conversion. The  
 photocurrent and photopotential generated by this cell were 70.0  $\mu$ A  
 and 888.0 mV, respectively. The effect of various parameters on the  
 electrical output of the cell was studied, and current-voltage  
 characteristics of the cell have also been observed.

11/9/10 (Item 1 from file: 144)

DIALOG(R)File 144:Pascal

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08896137 PASCAL No.: 90-0064116

**Use of the thionine - EDTA system in photogalvanic cells for solar  
 energy conversion**

AMETA S C; SADHNA KHAMESRA; SUSHILA LODHA; RAMESHWER AMETA

Sukhadia univ., dep. chemistry, Udaipur 313 001, India

Journal: Journal of photochemistry and photobiology. A. Chemistry, 1989  
 , 48 (1) 81-86

ISSN: 531731 CODEN: JPPCEJ Availability: CNRS-15990A

No. of Refs.: 19 ref.

Document Type: P (Serial) ; A (Analytic)

Country of Publication: Switzerland

Language: English

Description d'une cellule solaire utilisant de la **thionine** comme

Bowman-Birk...

...difference, other conditions were as in Fig. 6.

Reduction After

Protein 20 min 120 min

**Purothionin** -P 15 32

DSG-1 22 38

Corn kernel trypsin

inhibitor 3 15

Bowman-Birk...

...CM-1 a-amylase inhibitors (147 and 210%, respectively) ; corn kernel trypsin inhibitor (424%-) ; and **purothionin** (82, 133, and 120% for the a,, al and # forms, respectively) , Glutaredoxin was ineffective...and ovomucoid inhibitor) . Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 300C for 2 hours. The concentrations of thioredoxin, NTR,, and NADPH were 0,024 mg/ml, 0,02 mg/ml, and 0.25 mM, respectively. With DTT as reductant,, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA** ) chromatography. Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography

The pullulanase inhibitor sample...

...30 mM Tris

HCl pH 7,5, containing 200 mM Na Cl and 1 mM **EDTA** ,

Fractions (3\*6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

15/3,KWIC/58 (Item 27 from file: 349)

DIALOG(R)File 349:PCT FULLTEXT

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00230900

#### **BIOCIDAL PROTEINS**

#### **PROTEINES BIOCIDES**

Patent Applicant/Assignee:

IMPERIAL CHEMICAL INDUSTRIES PLC,

BROEKAERT Willem Frans,

CAMMUE Bruno Philippe Angelo,

OSBORN Rupert William,

REES Sarah Bronwen,

TERRAS Franky Raymond Gerard,

VANDERLEYDEN Jozef,

Inventor(s):

BROEKAERT Willem Frans,

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OSBORN Rupert William,

REES Sarah Bronwen,

TERRAS Franky Raymond Gerard,

VANDERLEYDEN Jozef,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9305153 A1 19930318

Application: WO 92GB1570 19920827 (PCT/WO GB9201570)

Priority Application: GB 9118523 19910829; GB 923038 19920213; GB 9213526 19920625

Designated States:



(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US AT BE CH  
DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD  
TG

Publication Language: English

Fulltext Word Count: 13654

Fulltext Availability:

Detailed Description

Claims

Detailed Description

... 1990

Eur J Biochem, 194:533-539). Such proteins, including SI=2 from sorghum and **purothionin** from wheat, are known to inhibit insect  $\alpha$ -amylase and are toxic to insect larvae...Rs-AFPI, Dm-AMP1. the Cb-AMPs, Lc-AFP, Ct-AMPI, sorghum Sia2, wheat Y1

**purothionin**, and the predicted products of the pea genes pl230 and pI39, of the cowpea gene...buffer containing 10 mM NaH<sub>2</sub>PO<sub>4</sub> VP 15, mM Na<sub>2</sub>HPO<sub>4</sub> 4J' 100 mM KCl 2 mM **EDTA**, 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...NaH PO 15 mM Na HPO 100 mM  
2 41 2 41

KClr 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8,3), 1% (w/v) SDS, 1 mM **EDTA**, 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dit loerythritol (DTE).

Proteins...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA**, The mixtures were allowed to react with 5,5t-dithionitrobenzoic acid and monitored for release...Sorghum

bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to  $\gamma$ - **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit 'in...

...the Cb-AMPs,

Lc-AFP, Ct-AMP1, the sorghum  $\alpha$ -amylase inhibitor SIa2, wheat yl **purothionin**, and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively, For the purpose of comparison, these tests were performed in parallel with 0- **purothionin**, an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...

...more than 30-fold with both test fungi,

in comparison, the IC<sub>50</sub> value of 0- **purothionin**

TABLE 6

VARIATIONS IN ANTIFUNGAL ACTIVITY IN THE PRESENCE OF X+ AND Fungus Antifungal IC<sub>50</sub>...

...6 10

Rs-AFP2 3 2 2 2

Rs-nsLTP 20 35 >1000 108

0- **purothionin** 10 7 4 10

Mj-AMP2 4 5 40 50

T hamatum Rs-AFP1 7...

...7 30

FULLTEXT AVAILABILITY:

| Available Text                     | Language  | Update | Word Count |
|------------------------------------|-----------|--------|------------|
| CLAIMS B                           | (English) | 200133 | 408        |
| CLAIMS B                           | (German)  | 200133 | 344        |
| CLAIMS B                           | (French)  | 200133 | 431        |
| SPEC B                             | (English) | 200133 | 5803       |
| Total word count - document A      |           |        | 0          |
| Total word count - document B      |           |        | 6986       |
| Total word count - documents A + B |           |        | 6986       |

...SPECIFICATION buffer containing 10 mM NaH<sub>2</sub>PO<sub>4</sub>), 15 mM Na<sub>2</sub>HPO<sub>4</sub>), 100 mM KCl, 2 mM **EDTA**, 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA**, 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and (beta)- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The (beta)- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and (beta)- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...

...g/ml for Mj-AMP2, from 0.5 to 15 (mu)g/ml for (beta)- **purothionin**, and from 20 to over 1,000 (mu)g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2 = (beta)- **purothionin** > Mj-AMP1 > UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to (beta)- **purothionin**. Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and (beta)- **purothionin** than for Mj-AMP1 or UDA. Also, Mj-AMP2 and (beta)- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. Figure 5 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and (beta)- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h ( \*----\* ), after 60...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. (beta)- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/67 (Item 3 from file: 348)  
 DIALOG(R) File 348:EUROPEAN PATENTS  
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00537586

Natural and synthetic proteins with inhibitory activity towards pathogenic microorganisms.

Natürliche und synthetische Proteine mit inhibitorischer Aktivität gegen pathogene Mikroorganismen.

Proteines naturelles et synthétiques avec activité inhibitrice contre des microorganismes pathogènes.

PATENT ASSIGNEE:

PIONEER HI-BRED INTERNATIONAL, INC., (1142071), 700 Capital Square 400 Locust Street, Des Moines Iowa 50309, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)

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LEGAL REPRESENTATIVE:

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PATENT (CC, No, Kind, Date): EP 502718 A1 920909 (Basic)

APPLICATION (CC, No, Date): EP 92301868 920304;

PRIORITY (CC, No, Date): US 664270 910304

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE

INTERNATIONAL PATENT CLASS: A01N-037/46; A01N-037/44; A01N-063/00;

A01N-065/00; C12N-015/00; C12N-015/52;

ABSTRACT WORD COUNT: 54

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

| Available Text | Language | Update | Word Count |
|----------------|----------|--------|------------|
|----------------|----------|--------|------------|

|          |           |        |     |
|----------|-----------|--------|-----|
| CLAIMS A | (English) | EPABF1 | 959 |
|----------|-----------|--------|-----|

|        |           |        |      |
|--------|-----------|--------|------|
| SPEC A | (English) | EPABF1 | 5135 |
|--------|-----------|--------|------|

|                               |      |
|-------------------------------|------|
| Total word count - document A | 6094 |
|-------------------------------|------|

|                               |   |
|-------------------------------|---|
| Total word count - document B | 0 |
|-------------------------------|---|

|                                    |      |
|------------------------------------|------|
| Total word count - documents A + B | 6094 |
|------------------------------------|------|

...SPECIFICATION oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...hormone 1-24, Citrate synthase, Defensin NPl, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Crothamine, Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.

Preferred proteins for...

...hormone 1-24, Citrate synthase, Defensin NPl, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.

Preferred proteins for killing...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NPl, a-hordothionin, b- **purothionin** and Melittin.

Preferred proteins for killing or inhibiting the pathogen

Colletotrichum graminicola are: Magainin-A...

...L-Lysine HCl, poly-D-Lysine, poly-D- Lysine HBr, Defensin NPl, a-hordothionin, b- **purothionin** and Melittin.

Preferred proteins for killing or inhibiting the pathogen Verticillium albo-atrum are: Magainin...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NPl, a-hordothionin, b- **purothionin** and Melittin.

Preferred proteins for killing or inhibiting the pathogen Phytophthora megaspermae f.sp. glycinea are: Magainin-2, poly-L-Lysine HBr, Defensin NPl, b- **purothionin** and Melittin.

Preferred proteins for killing or inhibiting the pathogen Macrophomina phaseolina are: poly-L-histidine, poly-D-Lysine, poly-D-Lysine HBr, Defensin NPl, a-hordothionin and b- **purothionin** .

Preferred proteins for killing or inhibiting the pathogen Diaporthe phaseolorum caulivora are: Defensin NPl, a-hordothionin and b- **purothionin** .

Preferred proteins for killing or inhibiting the pathogen Sclerotinia sclerotiorum are: Magainin-A, Magainin-G...

...poly-D-Lysine HBr, Mastoparan, Defensin NPl, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , stinging nettle lectin, Crothamine, Melittin, Eosinophil major basic protein and Eosinophil cationic protein.

Preferred proteins...

[Previous Doc](#)[Next Doc](#)  
[First Hit](#)[Go to Doc#](#)

Generate Collection

L5: Entry 27 of 28

File: DWPI

May 15, 2003

DERWENT-ACC-NO: 2003-585564

DERWENT-WEEK: 200355

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TITLE: Bacterial composition used for sterilizing food poisoning bacteria, includes ethylenediaminetetraacetic acid or its metal salts and alpha- or beta-type thionin

Basic Abstract Text (1):

NOVELTY - A bacterial composition comprises EDTA or its metal salts and alpha - or beta -type thionin.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

Publishing Model Print  
Document type: Journal Article  
Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: MEDLINE; Completed  
Subfile: INDEX MEDICUS

The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.

Tags: Comparative Study

Descriptors: \*Cartilage, Articular--cytology--CY; \*Methacrylates; \*Paraffin Embedding; \*Phenothiazines--metabolism--ME; Aged; Cartilage, Articular--anatomy and histology--AH; Chondroitin Lyases--metabolism--ME; Decalcification Technique; Glycosaminoglycans--metabolism--ME; Humans; Phenazines; Plastic Embedding; Sensitivity and Specificity; Staining and Labeling--methods--MT

CAS Registry No.: 0 (Glycosaminoglycans); 0 (Methacrylates); 0 (Phenazines); 0 (Phenothiazines); 477-73-6 (safranin T); 581-64-6 (thionine); 868-77-9 (hydroxyethyl methacrylate)

Enzyme No.: EC 4.2.2.- (Chondroitin Lyases)

Record Date Created: 19930412

Record Date Completed: 19930412

11/9/3 (Item 3 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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05131256 PMID: 594487

[Selective photooxidation of methionine, tyrosine and tryptophane using thionine and toluidine blue as sensitizers (author's transl)]

Fotooxidacion selectiva de metionina, tirosina y triptofano utilizando tionina y azul de toluidina como fotosensibilizadores.

Iborra J L; Llorca F I; Pastor R F; Garcia J V

Revista espanola de fisiologia (SPAIN) Dec 1977, 33 (4) p297-304,

ISSN 0034-9402 Journal Code: 0404475

Publishing Model Print

Document type: Journal Article ; English Abstract

Languages: SPANISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

**Thionine** and toluidine blue were used as sentizers on photooxidation processes of methionine, tryosine and tryptophane. They were more effective

than methylene blue. Methionine was photooxidized to sulfoxide and tryptophane to kinurenine. A tyrosine-sensitizer addition compound was postulated. Dye concentration, pH, temperature and EDTA presence conditions were determined on each one of the modification reactions. Methionine at acid pH was selectively modified. On the basis of obtained results and published references, a direct interaction of singlet oxygen with methionine and tryptophane and the excited dye with tyrosine was respectively discussed.

Tags: Comparative Study

Descriptors: \*Amino Acids--metabolism--ME; \*Photosynthesis--drug effects--DE; \*Tyrosine--metabolism--ME; Ergothioneine--pharmacology--PD; Methionine--metabolism--ME; Oxidation-Reduction--drug effects--DE; Photic Stimulation; Tolonium Chloride--pharmacology--PD; Tryptophan--metabolism--ME

CAS Registry No.: 0 (Amino Acids); 497-30-3 (Ergothioneine); 55520-40-6 (Tyrosine); 63-68-3 (Methionine); 73-22-3 (Tryptophan); 92-31-9 (Tolonium Chloride)

Record Date Created: 19780223

Record Date Completed: 19780223

11/9/4 (Item 4 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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04488011 PMID: 806084

**Mechanism of photoreduction of thiazine dyes by EDTA studied by flash photolysis III. Consequences of a newly found pKT of thionine on the mechanism in basic solutions.**

Bonneau R; Pereyre J

Photochemistry and photobiology (ENGLAND) Mar 1975, 21 (3) p173-7, ISSN 0031-8655 Journal Code: 0376425

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Descriptors: \*Dyes; \*Edetic Acid; \*Light; \*Oxidation-Reduction; \*Phenothiazines; \*Photolysis; \*Thiazines--metabolism--ME; Imines

CAS Registry No.: 0 (Dyes); 0 (Imines); 0 (Phenothiazines); 0 (Thiazines); 60-00-4 (Edetic Acid)

Record Date Created: 19750829

Record Date Completed: 19750829

11/9/5 (Item 1 from file: 5)

DIALOG(R) File 5:Biosis Previews(R)

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0003236407 BIOSIS NO.: 198171055366

**CHARACTERIZATION OF THE RED LIGHT INDUCED REDUCTION OF A PARTICLE**

**ASSOCIATED B TYPE CYTOCHROME FROM CORN IN THE PRESENCE OF METHYLENE BLUE**

AUTHOR: WIDELL S (Reprint); BRITZ S J; BRIGGS W R

AUTHOR ADDRESS: DEP PLANT PHYSIOL, BOX 7007, S-22007, LUND 7, SWEDEN\*\*  
SWEDEN

JOURNAL: Photochemistry and Photobiology 32 (5): p669-678 1980

ISSN: 0031-8655

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Methylene blue transfers electrons to a membrane-associated b-type cytochrome in particulate fractions from corn coleoptiles. The Km for methylene blue is less than 1 .mu.M under optimal conditions. This reaction is destroyed by boiling, but not by 7 M urea. Kinetic analyses of the influence of light intensity on cytochrome reduction suggest that

a 1st order photochemical reaction is limiting. Free **EDTA** may serve as an electron donor in this system at least at high methylene blue and protein concentrations. The photoactivity does not coincide either with mitochondrial or endoplasmic reticulum markers, and may be localized in plasma membrane. There is an estimated 5 .times. 10<sup>-10</sup> mol photoreducible cytochrome/g coleoptile tissue. Studies on the effect of pH on the reaction in the presence of methylene blue or **thionine** indicate that dye photoreduction itself is not rate-limiting. Wavelength dependence studies suggest that it is methylene blue monomer and not dimer which mediates the reaction. Although O<sub>2</sub> is apparently required for the reaction, neither superoxide nor excited singlet oxygen appear to be involved. The reaction mechanism is still unknown.

REGISTRY NUMBERS: 61-73-4: METHYLENE BLUE; 60-00-4: **EDTA** ; 7782-44-7: OXYGEN

DESCRIPTORS: COLEOPTILES ELECTRON TRANSFER **EDTA** WAVELENGTH DEPENDENCE OXYGEN REQUIREMENT

DESCRIPTORS:

MAJOR CONCEPTS: Bioenergetics--Biochemistry and Molecular Biophysics; Enzymology--Biochemistry and Molecular Biophysics; Radiation Biology

BIOSYSTEMATIC NAMES: Gramineae--Monocotyledones, Angiospermae, Spermatophyta, Plantae

COMMON TAXONOMIC TERMS: Angiosperms; Monocots; Plants; Spermatophytes; Vascular Plants

CHEMICALS & BIOCHEMICALS: METHYLENE BLUE; **EDTA** ; OXYGEN

CONCEPT CODES:

10012 Biochemistry - Gases

10060 Biochemistry studies - General

10064 Biochemistry studies - Proteins, peptides and amino acids

10065 Biochemistry studies - Porphyrins and bile pigments

10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation

10604 External effects - Light and darkness

10802 Enzymes - General and comparative studies: coenzymes

51516 Plant physiology - Light and radiation effects

52504 Agronomy - Grain crops

BIOSYSTEMATIC CODES:

25305 Gramineae

11/9/8 (Item 3 from file: 34)

DIALOG(R) File 34:SciSearch(R) Cited Ref Sci

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01233933 Genuine Article#: GG799 Number of References: 91

Title: MODEL PHOTOGALVANIC SYSTEMS FOR THE CONVERSION OF SOLAR-ENERGY

Author(s): SHAGISULTANOVA GA; TAITTS AM; TIMONOV AM

Corporate Source: AI GERTSEN STATE TEACHERS INST/LENINGRAD//USSR/

Journal: JOURNAL OF APPLIED CHEMISTRY OF THE USSR, 1990, V63, N12, P 2427-2440

Language: ENGLISH Document Type: ARTICLE

Geographic Location: UNION OF SOVIET SOCIALIST REPUBLICS

Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth Sciences

Journal Subject Category: CHEMISTRY, APPLIED

Identifiers--KeyWords Plus: **THIONINE** -COATED ELECTRODE;

**ETHYLENEDIAMINETETRAACETIC** ACID SYSTEM; PHOTO-CHEMICAL MECHANISMS; PHOTOELECTROCHEMICAL CELL; METHYLENE-BLUE; RATE CONSTANT; IRON SYSTEM; POWER; EFFICIENCY; KINETICS

Research Fronts: 89-6804 002 (PHOTOGALVANIC CELL FOR SOLAR-ENERGY CONVERSION; PHENOSAFRANIN DYE; METHYLENE BLUE-NITRILOTRIACETIC ACID SYSTEM)

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 QUICKENDEN TI, 1979, V24, P143, ELECTROCHIM ACTA  
 QUICKENDEN TI, 1985, V132, P81, J ELECTROCHEM SOC  
 QUICKENDEN TI, 1979, V83, P2496, J PHYS CHEM-US  
 QUICKENDEN TI, 1980, V84, P870, J PHYS CHEM-US



photosensibilisant et l' **EDTA** comme reducteur: caracteristiques potentiometriques en fonction de la concentration en **thionine** , en **EDTA** , du pH et de la geometrie de la couche et mecanismes de reaction.

English Descriptors: Solar cell; Energy conversion; Electrical characteristic; Photosensitizer; Medium effect; Potentiometry; Chemical concentration; Experimental study; Reaction mechanism; **EDTA**  
French Descriptors: Cellule solaire; Conversion energie; Caracteristique electrique; Photosensibilisant; Effet milieu; Potentiometrie; Concentration chimique; Etude experimentale; Mecanisme reaction; **EDTA** ; **Thionine**

Classification Codes: 230C02B; 001D06C02

11/9/11 (Item 2 from file: 144)  
DIALOG(R)File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

05746232 PASCAL No.: 84-0247280  
**Electrochemistry and photoelectrochemistry of dye-incorporated clay-modified electrode**  
KAMAT P V  
Univ. Notre Dame, radiation lab., Notre Dame IN 46556, USA  
Journal: Journal of electroanalytical chemistry and interfacial electrochemistry, 1984, 163 (1-2) 389-394  
ISSN: 0022-0728 Availability: CNRS-1150  
No. of Refs.: 13 ref.  
Document Type: P (Serial) ; A (Analytic)  
Country of Publication: Switzerland  
Language: English  
Etudes dans le cas de **thionine** incorporee dans des couches minces d'argile fixees sur des electrodes de SnO SUB 2 et de Pt. Resultats obtenus par etudes de voltammetrie cyclique, ainsi que donnees sur la generation de l'effet photoelectrochimique sous l'effet d'un rayonnement de  $\lambda > 460$  nm et en milieu aqueux contenant de l' **EDTA** 0,2 M

English Descriptors: Photoelectrochemistry; Semiconductor materials; Electrodes; Transition metal; Metal Oxides; Platinum; Thin layer electrode; Modified material; Chemical uptake; Thiazine dye; Electrochemical reaction; Cyclic voltammetry; Photoelectrochemical reaction; Aqueous solution; Organic solvent; Tin IV Oxides; Clay

French Descriptors: Photoelectrochimie; Semiconducteur; Electrode; Metal transition; Metal Oxyde; Platine-ACT; Electrode couche mince; Matériau modifié; Fixation chimique; Colorant thiazinique; Reaction electrochimique; Voltammetrie cyclique; Reaction photoelectrochimique; Solution aqueuse; Solvant organique; Etain IV Oxyde-ACT; Argile; **Thionine** ; Violet Lauth; **EDTA** -SUB

Classification Codes: 001C01H06

11/9/13 (Item 4 from file: 144)  
DIALOG(R)File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

04862032 PASCAL No.: 83-0108558  
**Effect of DNA and other polyanions on the EDTA induced photoreduction of thionine**  
MEDINI KANTA PAL; MANNA P C  
Univ. Kalyani, dep. chemistry, Kalyani 741235, India  
Journal: Makromol. Chem., 1982, 183 (11) 2811-2821  
ISSN: 0025-116X Availability: CNRS-4111  
No. of Refs.: 12 ref.

Document Type: P (Serial) ; A (Analytic)  
Country of Publication: Switzerland  
Language: English  
Influence de l'ADN, de l'ARN et du polystyrenesulfonate

English Descriptors: DNA; Polyanion; Reduction; Photochemical reaction;  
Styrenesulfonate polymer; RNA; Basic dye

French Descriptors: DNA; Polyanion; Reduction; Reaction photochimique;  
Styrenesulfonate polymere; RNA; Colorant basique; **Thionine** -ENT;  
Tetracetique acide( **ethylenediamine** ); Utilisation

Classification Codes: 780A02E

11/9/14 (Item 5 from file: 144)  
DIALOG(R)File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

04003372 PASCAL No.: 75-0028838

**MECHANISM OF PHOTOREDUCTION OF THIAZINE DYES BY EDTA STUDIED BY FLASH  
PHOTOLYSIS. III. CONSEQUENCES OF A NEWLY FOUND PK SUB T OF THIONINE ON  
THE MECHANISM IN BASIC SOLUTIONS**

BONNEAU R; PEREYRE J

LAB. CHIM. PHYS. A, UNIV. BORDEAUX I, 33405 TALENCE, FRANCE

Journal: PHOTOCHEM. AND PHOTOBIOLOG., 1975, 21 (3) 173-177

Availability: CNRS-9410

No. of Refs.: 13 REF.

Document Type: P (SERIAL) ; A (ANALYTIC)

Country of Publication: UNITED KINGDOM

Language: ENGLISH

EQUILIBRE ENTRE LES FORMES NEUTRE ET MONOCATIONIQUE DE LA **THIONINE**  
TRIPLET, SUP 3 T ET SUP 3 TH SUP + (PK SUB T =8,9). PAS D'EQUILIBRE  
IDENTIQUE AVEC LE BLEU DE METHYLENE. DIFFERENCE DUE A LEUR PHOTOREDUCTION  
DIFFERENTE PAR **EDTA**. LE RENDEMENT QUANTIQUE DE FORMATION DU COLORANT SEMI  
REDUIT EST PROPORTIONNEL AU RENDEMENT DE REDUCTION GLOBAL. MECANISMES

English Descriptors: METHYLENE BLUE; ORGANIC DYE; MONOCYCLIC COMPOUND;  
ACIDITY CONSTANT; **EDTA**; SULFUR NITROGEN HETEROCYCLE; REACTION MECHANISM  
; FLASH PHOTOLYSIS; PHOTOCHEMICAL REACTION; REDUCTION; QUANTUM YIELD  
English Generic Descriptors: PHYSICAL CHEMISTRY; PHOTOCHEMISTRY

French Descriptors: COLORANT ORGANIQUE; THIAZINE; BLEU METHYLENE; **THIONINE**  
; HETEROCYCLE SOUFRE AZOTE; COMPOSE MONOCYCLIQUE; **EDTA**; REDUCTION;  
REACTION PHOTOCHEMIE; CONSTANCE ACIDITE; RENDEMENT QUANTIQUE; PHOTOLYSE  
ECLAIR; MECANISME REACTION

French Generic Descriptors: CHIMIE PHYSIQUE; PHOTOCHEMIE

Classification Codes: 170A14B02A

11/9/22 (Item 13 from file: 144)  
DIALOG(R)File 144:Pascal  
(c) 2005 INIST/CNRS. All rts. reserv.

00112095 PASCAL No.: 73-0032420

**INDICADORES FOTO-REDOX EN AMPEROMETRIA. DETERMINACIONES INDIRECTAS DE  
ANIONES CON ETILENODIAMINO TETRAACETO DIHIDROGENO DISODICO  
(INDICATEURS PHOTO-REDOX EN AMPEROMETRIE. DOSAGE INDIRECT DES ANIONS AVEC  
L' EDTA -H SUB 2 NA SUB 2 )**

SIERRA F; SANCHEZ-PEDRENO C; PEREZ RUIZ T; MARTINEZ LOZANO C

C.S.I.C., DEP. QUIM. ANAL., DE MURCIA

Journal: INFORM. QUIM. ANAL., 1973, 27 (2) 93-98

Availability: CNRS-2711

No. of Refs.: 6 REF.

Document Type: P (SERIAL)

Country of Publication: SPAIN  
Language: SPANISH Summary Language: ENGLISH  
EMPLOI DE **THIONINE** COMME INDICATEUR REDOX-PHOTOCHIMIQUE POUR LE TITRAGE  
INDIRECT DE CRO SUB 4 SUP 2- , SO SUB 4 SUP 2- , PO SUB 4 H SUB 2 SUP - ET  
(FE(CN) SUB 6 ) SUP 4-

English Descriptors: AMPEROMETRY; CHEMICAL ANALYSIS; CHEMICAL COMPOSITION;  
CHROMATES; ANIONIC COMPLEX; **EDTA** ; ANALYTICAL INDICATOR; REDOX INDICATOR  
; PHOSPHATES; ABSORPTION SPECTROMETRY; ABSORPTION SPECTROSCOPY; SULFATES  
English Generic Descriptors: ANALYTICAL CHEMISTRY  
French Descriptors: **THIONINE** ; INDICATEUR ANALYTIQUE; CHROMATE; SULFATE;  
PHOSPHATE; FER II COMPOSE; FER II COMPLEXE; COMPLEXE CYANO; COMPLEXE  
ANIONIQUE; ANALYSE CHIMIQUE; AMPEROMETRIE; TITRAGE AMPEROMETRIQUE; **EDTA**  
; INDICATEUR REDOX; SPECTROMETRIE ABSORPTION; ANALYSE PAR; METHODE  
INDIRECTE; CYANO; ANALYSE  
French Generic Descriptors: CHIMIE ANALYTIQUE

Classification Codes: 170C05B06

11/9/51 (Item 3 from file: 434)  
DIALOG(R) File 434: SciSearch(R) Cited Ref Sci  
(c) 1998 Inst for Sci Info. All rts. reserv.

01388440 Genuine Article#: CW889 Number of References: 20  
Title: DETERMINATION OF IODIDE TRACES USING INHIBITORY EFFECT IN  
PHOTOCHEMICAL INTERACTION OF THIONINE AND ETHYLENEDIAMINETETRAACETIC  
ACID  
Author(s): SIERRA F; SANCHEZPEDRENO C; PEREZRUIZ T; MARTINEZLOZANO C;  
HERNANDEZCORDOBA M  
Corporate Source: FAC CIENCIAS MURCIA, CSIC, CTR COORDINADO, DEPT QUIM  
ANAL/MURCIA//SPAIN/  
Journal: ANALES DE QUIMICA, 1977, V73, N1, P67-70  
Language: SPANISH Document Type: ARTICLE  
Geographic Location: SPAIN  
Subfile: SciSearch; CC PHYS--Current Contents, Physical, Chemical & Earth  
Sciences  
Journal Subject Category: CHEMISTRY  
Cited References:

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CHANEY AL, 1940, V12, P179, IND ENG CHEM ANALYT  
DUBRAVCIC M, 1955, V80, P146, ANALYST  
ESPENSON JH, 1964, V86, P5101, J AM CHEM SOC  
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JOUSSOTDUBIEN J, 1963, V91, P1214, J CHIM PHYS  
KAZUO S, 1969, P119, TOHUKU U SERV  
LEIN A, 1951, V23, P1507, ANALYT CHEMISTRY  
MASSAE N, 1969, V42, P2464, B CHEM SOC JAPAN  
OSTER G, 1957, V79, P4836, J AM CHEM SOC  
SANDELL EB, 1934, V56, P1426, J AM CHEM SOC  
SIERRA F, 1972, V68, P1091, AN QUIMICA  
SIERRA F, 1974, V70, P595, AN QUIMICA  
SIERRA F, 1975, V78, P498, ANAL CHIM ACTA  
SIERRA F, 1975, V78, P277, ANALYT CHIM  
SIERRA F, 1971, V25, P73, INFORM QUIM ANAL  
SIERRA F, 1973, V27, P93, INFORM QUIM ANAL  
SIERRA F, TO BE PUBLISHED

?t s11/3,kwic/25-48  
>>>KWIC option is not available in file(s): 399

11/3,KWIC/25 (Item 1 from file: 399)  
DIALOG(R) File 399: CA SEARCH(R)  
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11/3,KWIC/30 (Item 6 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

106226561 CA: 106(26)226561c JOURNAL  
**Thionine blue as a photoredox indicator in titrations with EDTA.  
Determinations of metal ions, mixtures, and anions**  
AUTHOR(S): Perez Ruiz, T.; Martinez Lozano, C.; Tomas, V.; Yague, E.  
LOCATION: Fac. Cienc. Quim. Mat., Univ. Murcia, Murcia, Spain,  
JOURNAL: Quim. Anal. (Barcelona) DATE: 1986 VOLUME: 5 NUMBER: 2  
PAGES: 164-79 CODEN: QUANEL ISSN: 0212-0569 LANGUAGE: Spanish

11/3,KWIC/31 (Item 7 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
(c) 2005 American Chemical Society. All rts. reserv.

101113892 CA: 101(14)113892q JOURNAL  
**Thionine and ferric chelate compounds as coupled mediators in microbial  
fuel cells**  
AUTHOR(S): Tanaka, Kazuko; Vega, Carmen A.; Tamamushi, Reita  
LOCATION: Inorg. Chem. Lab., Inst. Phys. Chem. Res., Wako, Japan, 351  
JOURNAL: Bioelectrochem. Bioenerg. DATE: 1983 VOLUME: 11 NUMBER: 4-6  
PAGES: 289-97 CODEN: BEBEBP ISSN: 0302-4598 LANGUAGE: English

11/3,KWIC/32 (Item 8 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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99178976 CA: 99(22)178976e JOURNAL  
**Photochemical energy conversion by a thiazine  
photosynthetic-photoelectrochemical cell**  
AUTHOR(S): Pan, R. L.; Bhardwaj, R.; Gross, E. L.  
LOCATION: Dep. Biochem., Ohio State Univ., Columbus, OH, 43210, USA  
JOURNAL: J. Chem. Technol. Biotechnol., Chem. Technol. DATE: 1983  
VOLUME: 33A NUMBER: 1 PAGES: 39-48 CODEN: JCTTDW ISSN: 0142-0356  
LANGUAGE: English

11/3,KWIC/33 (Item 9 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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98071284 CA: 98(9)71284e JOURNAL  
**Effect of DNA and other polyanions on the EDTA induced photoreduction of  
thionine**  
AUTHOR(S): Pal, Medini Kanta; Manna, Pravash C.  
LOCATION: Fac. Sci., Univ. Kalyani, Kalyani, 741235, India  
JOURNAL: Makromol. Chem. DATE: 1982 VOLUME: 183 NUMBER: 11 PAGES:  
2811-21 CODEN: MACEAK ISSN: 0025-116X LANGUAGE: English

11/3,KWIC/34 (Item 10 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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93060423 CA: 93(6)60423v JOURNAL  
**The use of thionine as a photoredox indicator in amperometric titrations  
of the mercury(I) ion with polyaminopolycarboxylated agents**  
AUTHOR(S): Sierra, F.; Cebrian, A.; Hidalgo de Cisneros, J. L. H.  
LOCATION: Dep. Quim. Anal., Fac. Cienc. Murcia, Murcia, Spain,  
JOURNAL: Afinidad DATE: 1979 VOLUME: 36 NUMBER: 364 PAGES: 515-17  
CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

11/3,KWIC/35 (Item 11 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**88145569 CA: 88(20)145569u JOURNAL**

**Determination of periodate with photoreduced thionine**

AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.

LOCATION: Dep. Anal. Chem., Univ. Murcia, Murcia, Spain

JOURNAL: Anal. Chim. Acta DATE: 1977 VOLUME: 94 NUMBER: 1 PAGES: 129-33 CODEN: ACACAM ISSN: 0003-2670 LANGUAGE: English

**11/3,KWIC/36 (Item 12 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**88083095 CA: 88(12)83095a JOURNAL**

**Determination of trace iodide by using its inhibiting effect on the photochemical reaction between thionine and EDTA**

AUTHOR(S): Sierra, F.; Sanchez-Pedreno, C.; Perez Ruiz, T.; Martinez Lozano, C.; Hernandez Cordoba, M.

LOCATION: Dep. Quim. Anal., CSIC, Murcia, Spain

JOURNAL: An. Quim. DATE: 1977 VOLUME: 73 NUMBER: 1 PAGES: 67-70 CODEN: ANQUBU LANGUAGE: Spanish

**11/3,KWIC/37 (Item 13 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**86162741 CA: 86(22)162741v JOURNAL**

**Photoinduced potentials by photoredox systems and the effects of the addition of EDTA**

AUTHOR(S): Kaneko, Masao; Yamada, Akira

LOCATION: Inst. Phys. Chem. Res., Wako, Japan

JOURNAL: Rikagaku Kenkyusho Hokoku DATE: 1976 VOLUME: 52 NUMBER: 6 PAGES: 210-15 CODEN: RKKHAO LANGUAGE: Japanese

**11/3,KWIC/38 (Item 14 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**85195209 CA: 85(26)195209k TECHNICAL REPORT**

**Evaluation of some thionine redox systems as potential regenerative photogalvanic batteries**

AUTHOR(S): Fine, Dwight A.; Fletcher, Aaron N.

LOCATION: Nav. Weapons Cent., China Lake, Calif.

JOURNAL: U. S. NTIS, AD Rep. DATE: 1976 NUMBER: AD-A021424 PAGES: 25 pp. CODEN: XADRCH LANGUAGE: English CITATION: Gov. Rep. Announce. Index (U. S.) 1976, 76(9), 121 AVAIL: NTIS

**11/3,KWIC/39 (Item 15 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**82154984 CA: 82(23)154984d JOURNAL**

**Photochemical determination of a new acid-base equilibrium of thionine in its triplet state. Application to the photoreactivity of thiazine dyes in aqueous solution**

AUTHOR(S): Bonneau, Roland; Pereyre, Josette; Jousset-Dubien, Jacques

LOCATION: Lab. Chim. Phys. A, Univ. Bordeaux I 351, Talence, Fr.

JOURNAL: Mol. Photochem. DATE: 1974 VOLUME: 6 NUMBER: 3 PAGES: 245-52 CODEN: MLPCBL LANGUAGE: English

DIALOG(R)File 399:CA SEARCH(R)

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**74008313 CA: 74(2)8313c JOURNAL**

**Photochemical reduction of thionine and other thiazine dyes by Co(II)EDTA complex in a heterogeneous system**

AUTHOR(S): Singhal, G. S.; Rabinowitch, Eugene

LOCATION: Chem. Dep., State Univ. New York, Albany, N. Y.

JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 53 NUMBER: 10 PAGES:

4109-10 CODEN: JCPSA6 LANGUAGE: English

**11/3,KWIC/46 (Item 22 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

(c) 2005 American Chemical Society. All rts. reserv.

**73051836 CA: 73(10)51836s JOURNAL**

**Formation and stabilization of high valence states in silver**

AUTHOR(S): Sanchez-Manzanares, Jose A.

JOURNAL: An. Univ. Murcia, Cienc. DATE: 1968 VOLUME: 26 NUMBER: 1-4

PAGES: 57-161 CODEN: AUMCB5 LANGUAGE: Spanish

**11/3,KWIC/47 (Item 23 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**72122159 CA: 72(24)122159r JOURNAL**

**Photopolymerization using methylene blue-reducing agent system and its application to photoelectrochemical cell**

AUTHOR(S): Kamiya, Nobuyuki; Okawara, Makoto

LOCATION: Res. Lab. Resour. Util., Tokyo Inst. Technol., Tokyo, Japan

JOURNAL: Kogyo Kagaku Zasshi DATE: 1969 VOLUME: 72 NUMBER: 12 PAGES: 2639-44 CODEN: KGKZA7 LANGUAGE: Japanese

**11/3,KWIC/48 (Item 24 from file: 399)**

DIALOG(R)File 399:CA SEARCH(R)

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**72084849 CA: 72(16)84849f JOURNAL**

**Photochemical reduction of thionine by cobalt(II) EDTA complex in water-ether emulsion**

AUTHOR(S): Srinivasan, V.; Rabinowitch, E.

LOCATION: Dep. of Bot., Univ. of Illinois, Urbana, Ill.

JOURNAL: J. Chem. Phys. DATE: 1970 VOLUME: 52 NUMBER: 3 PAGES: 1165-8

CODEN: JCPSA6 LANGUAGE: English

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\$2.06 Estimated cost File5

\$0.74 0.033 DialUnits File34

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\$13.60 Estimated cost File34

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\$0.05 Estimated cost File35

\$0.06 0.011 DialUnits File48

\$0.06 Estimated cost File48

\$0.04 0.011 DialUnits File65

\$0.04 Estimated cost File65



US 20040005682A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0005682 A1**  
Chen et al. (43) **Pub. Date: Jan. 8, 2004**

(54) **BIOCIDAL PROTEIN**

**Related U.S. Application Data**

(76) **Inventors:** Ching-San Chen, Taipei (TW);  
Kuan-Chung Chen, Changhua City  
(TW); Cheng-Chun Kuan, Taipei  
(TW); Ching-Yu Lin, I-Lan (TW)

(62) Division of application No. 09/686,332, filed on Oct.  
11, 2000.

**Publication Classification**

**Correspondence Address:**  
**FISH & RICHARDSON PC**  
**225 FRANKLIN ST**  
**BOSTON, MA 02110 (US)**

(51) **Int. Cl.<sup>7</sup>** ..... C12N 9/99; C07H 21/04;  
C07K 14/47; C12P 21/02;  
C12N 5/06  
(52) **U.S. Cl.** ..... 435/184; 435/69.2; 435/320.1;  
435/325; 536/23.2; 530/358

(21) **Appl. No.:** 10/409,818

(22) **Filed:** Apr. 8, 2003

(57) **ABSTRACT**

The invention relates to novel nucleic acid and protein sequences from the mung bean *Vigna radiata*. The nucleic acid sequence, isolated from a bruchid resistant mung bean line, encodes a thionin-like protein with insecticidal properties.

## BIOCIDAL PROTEIN

### RELATED APPLICATIONS

[0001] This application is a divisional application and claims priority to U.S. application Ser. No. 09/686,332, filed Oct. 11, 2000, the contents of which are incorporated herein by reference.

### BACKGROUND OF THE INVENTION

[0002] Advances in biotechnology have enabled the generation of plants which express recombinant proteins. Thus, plants can be engineered to overproduce a variety of polypeptides with desirable qualities. Such polypeptide can include enzymes which produce secondary metabolites, proteins with medicinal or pharmaceutical properties, and proteins which endow the plants with new traits, for example, resistance to diseases, pathogens, and environmental conditions.

[0003] Given the vulnerability of agricultural crops to damage by insects, and other pests and pathogens, the ability to provide additional protective means and agents is of considerable importance. Moreover, traditional breeding techniques have identified plant lines with Mendelian traits endowing resistance to pests and pathogens. Modern molecular biological techniques can now be applied to isolate the critical nucleic acids and proteins with these properties in order to enhance the resistance of more sensitive plants or to antagonize the pests and pathogens in a variety of scenarios.

### SUMMARY OF THE INVENTION

[0004] The invention is based, in part, on the discovery of a novel mung bean nucleic acid which is expressed in a mung bean plant line that is resistant to insect attack, but not expressed in sensitive plant lines. The nucleic acid encodes a polypeptide which has insecticidal activity and which has similarity to thionin proteins. The sequence of the mung bean thionin nucleic acid (SEQ ID NO: 1), designated as "VrCRP", is shown below:

5'-ACCTCAACAATTCATCACTCATGGAGAGAAAACTTTCAGCTTCTTG  
TTCTCGCTCCTTCTCGTCTTAGCCTCTGATGTGGCCGTAGAGAGAGAGA  
GGCTAGAACTTGATGATAAAGAAAGAGGGTGGGGAAAATGCTTAATTG  
ACACCACCTGTGCACATTCGTGCAAGAACCGCGTTACATAGGTGGAGAT  
TGCAAGGCGATGACGCGCACCTGCTATTGCCTCGTCAACTGTTGACCCCT  
TTTCGAATATCATATCATCTTATCACAATAAATATAGCAGCATCACTGC  
TACTAGTACCGCCCTCCGCACCAACGCCCT-3'

[0005] The initiator and terminator codons are underlined and in boldface. The sequence of the mung bean thionin polypeptide sequence (SEQ ID NO:2), designated as "VrCRP", is shown below:

MERKTFSLPLSLLVLVLSDAVGEARTCMIKKEGNGKCLIDTTCASHC  
KNRGYIGDCKGMTRTCTCYCLVNC

[0006] The invention is also based on the discovery of a polypeptide derived from VrCRP which has the VrCRP

signal sequence removed is biologically active as an insecticide and fungicide. This polypeptide is encoded by the nucleic acid sequence (SEQ ID NO:3) below:

GAGAGAGGAGAGGCTAGAACTTGATGATAAAGAAAGGGTGGGAAA  
ATGCTTAATTGACACCACCTGTGCACATTCGTGCAAGAACCGCGTTACA  
TAGGTGGAGATTGCAAGGCGATGACGCGCACCTGCTATTGCCTCGTCAAC  
TGTTGA

[0007] The polypeptide sequence (SEQ ID NO:4) of this form of VrCRP lacking the signal sequence is shown below:

ERGZARTCMIKKEGNGKCLIDTTCASHCKNRGYIGDCKGMTRTCTCYCLV  
NC

[0008] Accordingly, in one aspect, the invention features isolated nucleic acid sequences which include a nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO: 1. The nucleic acids of the invention can further include nucleic acids which hybridize under stringent conditions to SEQ ID NO:1, as well as nucleic acids which are at least 50% identical, e.g., at least 60%, 70%, 80%, 90%, or 95% identical, to SEQ ID NO: 1. Such nucleic acid sequences can encode a polypeptide which inhibits translation of messenger RNAs in a wheat germ extract, a polypeptide which has insecticidal activity, e.g., insecticidal activity against bruchids such as *Callosobruchus chinensis*, *Callosobruchus maculatus*, and *Zabrotes subfasciatus*, or a polypeptide which has anti-fungal activity, e.g., against *Rhizoctonia solani*.

[0009] In another aspect, the invention features polypeptides comprising the amino acid sequence of SEQ ID NO:2. Featured polypeptides also include polypeptides which are at least 50% identical, e.g., at least 60%, 70%, 80%, 90%, or 95% identical, to SEQ ID NO:2. Such polypeptides can have at least one, two, three, four, five, eight, ten, twelve, or twenty conservative amino acids substitutions. The polypeptides can inhibit translation of messenger RNAs in a wheat germ extract, can have insecticidal activity, e.g., insecticidal activity against bruchids such as *Callosobruchus chinensis*, *Callosobruchus maculatus*, and *Zabrotes subfasciatus*, or can have anti-fungal activity, e.g., against *Rhizoctonia solani*. Also encompassed by the invention are nucleic acid sequences encoding such polypeptides.

[0010] The featured polypeptides can be recombinant and/or purified. For example, they can be overexpressed in a variety of host cells, such as *E. coli*, Sf9 insect cells, plant cells and mammalian tissue culture cells using overexpression vectors known in the art. Lysates are made from the host cells, e.g., after overexpression is induced if induction is required. The polypeptides are purified from the lysate. Alternatively, the polypeptides are secreted, by the inclusion nucleic acid sequences encoding the signal peptide or a heterologous signal peptide. In another example, the featured polypeptides are encoded by a transgene and overproduced in a plant or a plant tissue. The plant is harvested and the polypeptides purified from the plant.

[0011] The purified and/or recombinant polypeptides can be formulated a composition. The composition can include an agriculturally acceptable carrier, e.g., one described



below. The composition can contain the polypeptide at a concentration of about 0.005% to 10%, or about 0.01% to 1%, or about 0.1% to 0.5% by weight of composition. The composition can further include a cyclopeptide alkaloid, e.g., vignatic acid A and vignatic acid B, e.g., a cyclopeptide alkaloid with insecticidal properties. The composition can also include other desirable compounds, e.g., protease inhibitors, endotoxins, and the like. The contemplated compositions can have insecticidal activity, e.g., against bruchids such as *Callosobruchus chinensis*, *Callosobruchus maculatus*, and *Zabrotes subfasciatus*, and/or anti-fungal activity, e.g., against *Rhizoctonia solani*. The compositions can be applied to plants and their environs by methods described below.

[0012] The nucleic acids of the invention can also include a heterologous promoter such that the promoter is operably linked to a coding genomic nucleic acid or a cDNA. The promoter can direct transcription of the nucleic acid in wounded or pathogen infected cells. The promoter can be induced by a signalling molecule, e.g., methyl jasmonate, salicylic acid, ethylene, abscisic acid, gibberellins,  $HgCl_2$ , and  $H_2O_2$ . The invention also features transformed cells which contain such nucleic acids, i.e., an aforementioned nucleic acid operably linked to a heterologous promoter. Also included are transgenic plants whose genomic DNA includes such nucleic acids, as are transgenic seeds from such plants.

[0013] A "purified polypeptide", as used herein, refers to a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated. The polypeptide can constitute at least 10, 20, 50, 70, 80 or 95% by dry weight of the purified preparation.

[0014] An "isolated nucleic acid" is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid, or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of different (i) DNA molecules, (ii) transfected cells, or (iii) cell clones: e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

[0015] The term "substantially pure" as used herein in reference to a given polypeptide means that the polypeptide is substantially free from other biological macromolecules. The substantially pure polypeptide is at least 75% (e.g., at least 80, 85, 95, or 99%) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0016] The "percent identity" of two amino acid sequences or of two nucleic acids is determined using the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-68, 1990, modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. *J. Mol. Biol.* 215:403-10, 1990. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the invention. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[0017] As used herein, the term "transgene" means a nucleic acid sequence (encoding, e.g., one or more subject thionin polypeptides), which is partly or entirely heterologous, i.e., foreign, to the transgenic plant or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic plant or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the plant's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of the selected nucleic acid, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

[0018] As used herein, the term "transgenic cell" refers to a cell containing a transgene. As used herein, a "transgenic plant" is any plant in which one or more, or all, of the cells of the plant includes a transgene. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by T-DNA mediated transfer, electroporation, or protoplast transformation. The transgene may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

[0019] As used herein, the term "tissue-specific promoter" means a DNA sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in specific cells of a tissue, such as a leaf, a root, or a stem.

[0020] As used herein, the term "hybridizes under stringent conditions" refers to conditions for hybridization in 6x sodium chloride/sodium citrate (SSC) at about 45° C., followed by one or more washes in 0.2x SSC, 0.1% SDS at 50-65° C.

[0021] A "heterologous promoter", when operably linked to a nucleic acid sequence, refers to a promoter which is not naturally associated with the nucleic acid sequence.

[0022] As used herein, an agent with "insecticidal activity" is an agent which when tested by the following assay

has measurable insect-killing activity. In the assay, the agent is combined with mung bean flour produced from an insect-sensitive mung bean strain and packed as an artificial bean. Insect eggs are placed on the artificial bean. The timing and number of hatched insects are measured. An agent with "insecticidal activity" demonstrates delayed hatching, e.g., a delay of about 2, 4, 5, 7, 10, or 14 days. Alternatively, the agent can prevent hatching, e.g., only about 80%, 60%, 40%, 30%, 10%, or 0% of the eggs hatch after 14 days.

[0023] As used herein, an agent with "fungicidal activity" is an agent which when tested in the following assay produces a measurable zone of growth inhibition. The agent, in an acceptable solvent, is soaked in a sterile filter disc which is placed on an agar plate top spread with a fungus, e.g., *Rhizoctonia solani*. The plates are incubated, e.g., for 36-48 hours, and then zone of growth inhibition around each filter disc is measured. An agent with "anti-fungal activity" produces a zone of inhibition of about 1, 2, 3, 4, 5 mm or greater after 36 hours of fungus growth.

[0024] As used herein, an agent which "inhibits messenger RNA translation in a wheat germ extract" is an agent which when present in an in vitro translation reaction obtained by combining a messenger RNA with a wheat germ extract, e.g., a commercial wheat germ extract, prevents incorporation of [35S] methionine into an acid insoluble fraction by at least 30%, e.g., at least about, 50%, 75%, or 100%.

[0025] The discovery of a polypeptide thionin from insect resistant mung beans with insecticidal properties and its encoding nucleic acid sequence has a variety of commercial and agriculture benefits. The polypeptide can be used in a composition, e.g., a composition which includes agriculturally acceptable carriers, or other agents, to protect plants from insects, and other pests and pathogens. The composition can be formulated and applied by methods described herein in order to protect a plant. In another aspect, the nucleic acid can be used to generate a transgenic plant which expresses the thionin to thereby protect the plant. By conferring resistance to damage by insects, these strategies are of considerable economic benefit.

[0026] Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

#### DETAILED DESCRIPTION

[0027] The invention provides nucleic acids and polypeptides with insecticidal properties. These molecules can be isolated from a bruchid resistant line of the mung bean *Vigna radiata*. Moreover, isolated nucleic acids and isolated polypeptides of the invention can be used to provide pest and pathogen resistance in a variety of scenarios.

#### [0028] Assaying Resistance

[0029] A simple bioassay is utilized in order to assess the insecticidal properties of the plant lines, nucleic acids, and polypeptides featured herein. Bruchids, (e.g., bruchids obtained from the Asian Vegetable Research and Development Center (AVRDC), P.O. Box 42, Shanhua, Tainan 741, Taiwan, are maintained on sensitive mung bean seeds, e.g., on VC1973A seeds. To assay mung bean plants for resistance, seeds are obtained from the plant in question. Six bruchid eggs are placed on a seed. Multiple seeds from a single plant in question can be so tested. The seeds are

incubated at 25° C. and 60% humidity, and observed daily. The number of live bruchids emerging each day is monitored, and compared to data obtained from control sensitive and resistant lines.

[0030] In order to determine if a composition, e.g., a formulation containing a polypeptide featured in this invention, has insecticidal properties, the above bioassay is easily adapted to testing the composition. Flour is produced from sensitive mung bean seeds, combined with the composition and molded into an artificial seed following the method of Shade et al.(1986) *Bio/Technology* 12:793-796. Again, six bruchid eggs are placed on each seed. The artificial seeds are monitored as the real seeds described above.

#### [0031] Polypeptide Expression

[0032] The nucleic acids featured herein can be utilized to express polypeptides with insecticidal properties. Methods for expressing and obtaining polypeptides from coding nucleic acid sequences are routine in the art. The coding nucleic acid sequence for mung bean thionin can be cloned into an expression vector, for example, a bacterial expression vector. The vector can have an inducible promoter, e.g., the lac promoter or a derivative thereof. Alternatively, the vector can have a T7 polymerase promoter. The vector can also include nucleic acid sequences encoding a polypeptide tag or fusion gene to facilitate purification of an inserted heterologous coding sequence. For example, the tag can be a short peptide epitope for an antibody, or a purification handle such as hexa-histidine. The tag can encode a completely folded polypeptide, such as glutathione-S-transferase, maltose binding protein, or chitin binding domain. Between the tag and the inserted coding sequence can be sequence encoding a site specific protease recognition site. The vector can include a sequence to export the desired polypeptide into the periplasm.

[0033] The vector is transformed into a host cell, e.g., *E. coli* DH5 $\alpha$ . Transformed cells can be propagated, and treated with an inducer to activate polypeptide expression.

[0034] If the expressed polypeptide is fused to a tag or fusion gene with a purification handle, the polypeptide can be easily purified from a clarified cell lysate with an appropriate affinity column, e.g., Ni<sup>2+</sup> NTA resin for hexa-histidine, glutathione agarose for GST, amylose resin for maltose binding protein, chitin resin for chitin binding domain, and antibody affinity columns for epitope tagged proteins. The desired polypeptide can be eluted from the affinity column, or if appropriate cleaved from the column with a site specific protease. If the protein is not tagged for purification, routine methods in the art can be used to develop procedures to isolate it from cell lysates, periplasm, or the media (see, e.g., Scopes, RK (1994) *Protein Purification: Principles and Practice*, 3rd ed., New York: Springer-Verlag).

[0035] Analogs of mung bean thionin include thionins (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish bruchid killing activity. The following table list suitable amino acid substitutions:

TABLE 1

| CONSERVATIVE AMINO ACID REPLACEMENTS |      |                     |
|--------------------------------------|------|---------------------|
| For Amino Acid                       | Code | Replace with any of |
| Alanine                              | A    | Gly, Ala, Cys       |
| Arginine                             | R    | Lys, Met, Ile,      |
| Asparagine                           | N    | Asp, Gln, Gln,      |
| Aspartic Acid                        | D    | Asn, Gln, Gln       |
| Cysteine                             | C    | Met, Thr            |
| Glutamine                            | Q    | Asn, Gln, Asp       |
| Glutamic Acid                        | E    | Asp, Asn, Gln       |
| Glycine                              | G    | Ala, Pro,           |
| Isoleucine                           | I    | Val, Leu, Met       |
| Leucine                              | L    | Val, Leu, Met       |
| Lysine                               | K    | Arg, Met, Ile       |
| Methionine                           | M    | Ile, Leu, Val       |
| Phenylalanine                        | F    | Tyr, His, Trp       |
| Proline                              | P    |                     |
| Serine                               | S    | Thr, Met, Cys       |
| Threonine                            | T    | Ser, Met, Val       |
| Tyrosine                             | Y    | Phe, His            |
| Valine                               | V    | Leu, Ile, Met       |

**[0036] Promoters**

**[0037]** For polypeptides which confer resistance to pests or pathogens, the isolated coding nucleic acid sequence can be operably linked to a heterologous promoter. The promoter can activate transcription and, thus, polypeptide expression in a subset of tissues. Alternatively, the promoter can alter transcription rates in response to environmental stimuli, systemic signals, or intracellular signals. For example, promoters are known which respond to excessive heat, tissue injury, pathogen infection, or cell wounding, e.g., wounding due to pest attack. Known signals for promoters included methyl jasmonate, abscisic acid, gibberellins, salicylic acid, ethylene,  $HgCl_2$ , and  $H_2O_2$ . Methyl jasmonate responsive promoters include vspB (Mason et al. (1993) *Plant Cell* 5:241-251), and the tomato HMG2 promoter (U.S. Pat. No. 5,689,056). An example of a gibberellin response promoter is the Amy1/6-4 promoter of rice (Skriver et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:7266-7270). Promoters which respond to pathogen infection include the grape stilbene synthase promoter (U.S. Pat. No. 6,072,103). Promoters which respond to cell wounding include the win1 and win2 promoters (Weiss and Bevan (1991) *Plant Physiol.* 96:943-951), and the PinII promoter (U.S. Pat. No. 5,684,239). For example, any of these promoters can be operably linked to a nucleic acid sequence of SEQ ID NO: 1 in order to regulate expression of the polypeptide of SEQ ID NO:2. Similarly, any of these promoters can be operably linked to variants or fragments of SEQ ID NO: 1, or other similar coding nucleic acid sequences.

**[0038] Methods of Transforming Plant Cells**

**[0039]** A nucleic acid construct of the present invention can be transformed into a plant cell to produce a desired transgenic plant or plant cell. Methods for transforming plant cells with nucleic acid are routine in the art. Further, the plant cells can be transformed with multiple constructs, e.g., sequentially or concurrently. Depending on the desired physiological and agronomic properties of a plant species, and the nucleic acid construct of the present invention, a target plant or plant cell for transformation can include a species from maize, wheat, rice, soybean, tomato, tobacco, carrots, peanut, potato, sugar beets, sunflower, yam, Arabidopsis, rape seed, sunflower, and petunia.

**[0040]** One implementation of the current invention utilizes *Agrobacterium*, to introduce the desired construct into plant cells such as in U.S. Pat. Nos. 5,177,010, 5,104,310, 5,149,645, 5,469,976, 5,464,763, 4,940,838, 4,693,976, 5,591,616, 5,231,019, 5,463,174, 4,762,785, 5,004,863, and 5,159,135; and European Patent Applications 1 6718, 290799, 320500, 604662, 627752, 0267159, and 0292435). The method can be used with both dicotyledonous plants cells (Bevan et al. (1982) *Ann. Rev. Genet.* 16:357-384; Rogers et al. (1986) *Methods Enzymol.* 118:627-641), and monocotyledonous plant cells. (Hermalsteen et al. (1984) *EMBO J* 3:3039-3041; Hooykass-Van Slogteren et al. (1984) *Nature* 311:763-764; Grimsley et al. (1987) *Nature* 325:1677-179; Boulton et al. (1989) *Plant Mol. Biol.* 12:31-40.; Gould et al. (1991) *Plant Physiol.* 95:426-434). The method employs binary *Agrobacterium* T-DNA vectors (Hoekema et al. (1983) *Nature* 03:179; Bevan, 1984, *Nuc. Acid Res.* 12:8711-8721), and the co-cultivation procedure (Horsch et al., 1985, *Science* 227:1229-1231).

**[0041]** Additional steps may be required to prepare a desired nucleic acid sequence for plant transformation. For example, in order to utilize T-DNA mediated transformation, the thionin coding sequence, operably linked to a heterologous promoter, is ligated into a binary vector, between the left and right border sequences of T-DNA. The binary vector further includes an Hph gene coding for hygromycin resistance. The binary vector containing the desired construction is transformed into an *E. coli* strain, e.g., DH5 $\alpha$ . Subsequently, the binary plasmid is transferred into an *Agrobacterium*, e.g., *Agrobacterium* strain LBA4404, using a tri-parental mating.

**[0042]** Meanwhile, plants are prepared to receive the T-DNA with the transgene. Leaf discs are obtained from axenically grown tobacco seedlings. The discs are incubated for 8 hours on sterile filter papers overlaid on tobacco nurse cells on a feeder plate containing modified MS medium with Nitsch vitamins, 100 ml/L myo-inositol, 30 mg/L sucrose, 0.4 mg/L BAP, 1 mg/L 2,4-D (dichlorophenoxyacetic acid), 8 ml/L agar. To establish co-cultivation, the filters bearing the leaf disks are submersed in a suspension of the *Agrobacterium* bearing the desired binary vector, the bacteria being a concentration of approximately  $1 \cdot 10^9$  cell/ml, and vacuum infiltrated (3x1 minute). The filters and leaf discs are incubated on the nurse plate for 48 hours at 25° C. with indirect light. Then the discs are transferred to selection/regeneration plates containing MS salts, Nitsch vitamins, 100 ml/L myo-inositol, 20 g/L sucrose, 2 mg/L zeatin, 4 g/L agar, 500  $\mu$ g/ml carbamicillin and an appropriate antibiotic, e.g., G418 to select for the hygromycin resistance gene. The plates are placed in a growth chamber at 25° C. for 18 hours with light. The resulting shoots were transferred to rooting media, grown into plantlets, transferred to soil, and grown into plants in a green house. One skilled in the art can adapt this method to transform other species of plants.

**[0043]** Other methods for transforming plant cells are available. Of particular utility for transforming monocotyledonous plants or plant cells are methods of protoplast transformation which include, but are not limited to, protoplast transformation through calcium-, polyethylene glycol (PEG)- or electroporation-mediated uptake of naked DNA (see Paszkowski et al., 1984, *EMBO J* 3:2717-2722, Potrykus et al. 1985, *Molec. Gen. Genet.* 199:169-177; Fromm

et al., 1985, *Proc. Nat. Acad. Sci. USA* 82:5824-5828; Shimamoto, 1989, *Nature* 338:274-276), microinjection, silicon carbide mediated DNA uptake (Kaeppler et al., 1990, *Plant Cell Reporter* 9:415-418), and microprojectile bombardment (see Klein et al., 1988, *Proc. Nat. Acad. Sci. USA* 85:4305-4309; Gordon-Kamm et al., 1990, *Plant Cell* 2:603-618), whiskers technology (see U.S. Pat. Nos. 5,302, 523 and 5,464,765), and viral vector systems (see, U.S. Pat. Nos. 5,316,931, 5,589,367, 5,811,653, and 5,866,785).

[0044] A transformed plant or transformed plant tissue can be assayed for resistance to pathogens, insects, and other pests (e.g., by a field trial or by a method described herein).

[0045] Agricultural Compositions

[0046] Polypeptides, e.g., mung bean thionin protein, can be formulated as a composition which is applied to plants in order to confer insect, pest, or pathogen resistance. The composition can be prepared in a solution, e.g., an aqueous solution, at a concentration from about 0.005% to 10%, or about 0.01% to 1%, or about 0.1% to 0.5% by weight of polypeptide content. The solution can comprise an organic solvent, e.g., glycerol or ethanol. Alternatively, the composition can be formulated with one or more agriculturally acceptable carriers. Agricultural carriers can include: clay, talc, bentonite, diatomaceous earth, kaolin, silica, benzene, xylene, toluene, kerosene, N-methylpyrrolidone, alcohols (methanol, ethanol, isopropanol, n-butanol, ethylene glycol, propylene glycol, and the like), and ketones (acetone, methyl ethyl ketone, cyclohexanone, and the like). The formulation can optionally further include a stabilizer, spreading agent, wetting extenders, dispersing agents, sticking agents, disintegrators, and other additives, and can be prepared as a liquid, a water-soluble solid (e.g., tablet, powder or granule), or a paste.

[0047] Prior to application, the solution can be combined with another desired composition such as an insecticide, germicide, fertilizer, plant growth regulator and the like. The solution may be applied to the plant tissue, for example, by spraying, e.g., with an atomizer, by drenching, by pasting, or by manual application, e.g., with a sponge. The solution can also be distributed from an airborne source, e.g., an aircraft or other aerial object, e.g., a fixture mounted with an apparatus for spraying the solution, the fixture being of sufficient height to distribute the solution to the desired plant tissues. Alternatively, the composition can be applied to plant tissue from a volatile or airborne source. The source is placed in the vicinity of the plant tissue and the composition is dispersed by diffusion through the atmosphere. The source and the plant tissue to be contacted can be enclosed in an incubator, growth chamber, or greenhouse, or can be in sufficient proximity that they can outdoors.

[0048] In another implementation, if the composition is distributed systemically thorough the plant, the composition can be applied to tissues other than the leaves, e.g., to the stems or roots. Thus, the composition can be distributed by irrigation. The composition can also be injected directly into roots or stems.

[0049] Without further elaboration, it is believed that the above description has adequately enabled the present invention. The following examples are, therefore to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All of the publications cited herein are hereby incorporated by reference in their entirety.

[0050] Materials and Methods

[0051] Plant and Insect Materials

[0052] Two nearly isogenic lines of mung bean *Vigna radiata* L. VC 1973A and VC6089A were obtained from the Asian Vegetable Research and Development Center (AVRDC). VC6089A (BC6S2) populations were crossed to wild mung bean *Vigna radiata* TC1966 as donor parent and breeding line VC1973A as recurrent parent. Azuki bean weevil *Callosobruchus chinensis* (L.) was also obtained from AVRDC that has been maintained on VC1973A at 25° C. in a growth chamber.

[0053] Isolations and Analyses of RNA and DNA

[0054] Total RNA was isolated via a modified hot phenol extraction method (Verwoerd et al. (1989) *Nucl Acids Res* 17:2362). Polyadenylated RNA was prepared by Oligo(dT)-cellulose affinity column (Maniatis et al. (1982) *Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.*). RNA concentration was determined by measuring its absorbance at 260 nm and verified by agarose gel electrophoresis and ethidium bromide staining. To confirm equal loading of total RNA on RNA gel blots, a 0.2 kb fragment of VrCRP was PCR amplified and used as a probe. Genomic DNA isolation was performed via a modified CTAB extraction method (Delaporta et al. (1983) *Plant Mo. Biol. Rep.* 1:19). DNA gel blot analysis, including restriction, fragment length polymorphism, and downward alkaline capillary transfer to a positively charged nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Baue D'Urfe, Canada) were performed as described by Young et al. ((1992) *Theor. Appl. Genet.* 84:839-844) and Chomczynski ((1992) *Anal. Biochem.* 201:134-139). Hybridization of the membrane was performed under high-stringency condition at 65° C. overnight, and after hybridization the membrane was washed twice with 2×SSC/1% SDS for 15 min at room temperature, and then washed twice with 0.2×SSC/1% SDS at 55° C. for 15 min (1×SSC is 0.15M NaCl, 0.015 M sodium citrate, pH 7.0). RNA gel blot analyses were also carried out as described by Chomczynski (1992) supra. RNAs were downward alkaline capillary-transferred onto Hybond N+ nylon membranes and cross-linked with a Stratalinker™ UV Crosslinker 1800. Hybridization and washing of the membranes was performed as described previously. Probes for both DNA and RNA gel blot analysis were synthesized from random-labeled isolated DNA inserts with  $\alpha$ -<sup>32</sup>P-dCTP. The membranes were exposed to phosphorimager, and scanned.

[0055] Suppression Subtractive Hybridization

[0056] Suppression subtractive hybridization (SSH) was performed on VC6089A cDNA and matched VC1973A cDNA, using the PCR select cDNA subtraction kit (Clontech Laboratories Inc., CA) according to the manufacturer's recommendations. VC6089A and VC1973A seeds were collected 15 DAF (days after flowering). mRNA was prepared from the seeds as described above, and reverse transcribed to generate cDNA, which is then converted to double-stranded DNA. The two cDNA populations were subtracted as described (Akopyants et al., ((1998) *Proc. Natl. Acad. Sci. USA* 95: 13108-13113). Briefly, the VC6089A cDNA, designated the "tester DNA", was digested with a restriction enzyme, e.g., Alul, and divided into two pools. Each pool was ligated to a different linker adaptor. The VC1973A DNA

was used as the "driver DNA," and was restricted with the same enzyme. Each pool of VC6089A cDNA was denatured and hybridized to an excess of digested driver DNA. Subsequently, the mixtures were cooled, and the DNA ends were extended in order to incorporate the adaptor end into the driver DNA strand. Finally, the two pools were combined, denatured, and reannealed. The ends of the reannealed strands were filled in with DNA polymerase. PCR amplification was used to amplify fragments that had two different linkers at each end, one from each pool. The procedure highly favors amplification of DNAs which were unable to anneal to DNA in the driver DNA population, i.e., cDNAs were more abundant in VC6089A seeds than VC1973A seeds.

[0057] The amplified DNAs were cloned into the pGEM-T vector, transformed into competent *E. coli* cells, and grown in 96-well dishes. Each clone was transferred to a nitrocellulose membrane for blot hybridization to identify vectors with inserts. Inserts were amplified by PCR using the universal primers T7 and SP6, sequenced, and used for Northern blot analysis against mRNA from sensitive (VC1973A) and resistant (VC6089A) seeds. One DNA fragments which was differentially expressed in VC6089A contains nucleic acid encoding VrCRP.

[0058] Sequencing of DNAs and Peptides

[0059] DNA sequencing was carried out using a ABI autosequencer 377. N-terminal amino acid sequences were determined by automatic Edman degradation method using a gas-phase protein sequencer (Applied Biosystems, Model 473A). After SDS-PAGE separation, polypeptides were electroblotted onto a PVDF membrane and detected by Coomassie blue staining. Bands corresponding to the desired polypeptides were cut and processed for amino acid sequencing.

[0060] Construction of an Expression Vector

[0061] Two degenerate oligonucleotides were synthesized: VrCRF (5'-CAT GCC ATG GAG AGA GGA GAG GCT AGA AC-3') and VrCRR (5'-TCC CCC GGG ACA GTT GAC GAG GCA ATA-3'). VrCRF corresponds to the degenerate codons of the amino acids Glu<sup>23</sup> to Arg<sup>28</sup> (N-terminal amino acid methionine of the signal peptide is numbered as amino acid 1) of VrCRP and has a sense orientation and a NcoI site. VrCRR corresponds to the degenerate codons of the amino acids Tyr<sup>68</sup> to Cys<sup>73</sup> of VrCRP and has an antisense orientation and a SmaI site. PCR was performed essentially by the method of DNA polymerase chain reaction (Saiki et al., 1988), using 5 ng of VrCRP as target DNA, 10 pmol each of VrCRR and VrCRF, 250 nmol each of the dNTPs and 2.5 U Taq polymerase (Promega) in a total volume of 100  $\mu$ L. The amplification program included an initial step at 95° C. for 5 min, 25 cycles (95° C. for 1 min, 55° C. for 1 min, and 72° C. for 1 min) and a final step at 72° C. for 10 min. The 171 bp amplification product was purified on a 1% agarose (FMC BioProducts) gel and excised from the gel with a razor blade (Chuang et al. (1994) *Biotechniques* 17:634-636). The sequence of the amplification product was confirmed by DNA sequencing. The amplification product and pTYB4 expression vector that has NcoI and SmaI sites on its multiple cloning sites (New England Biolabs, MA) were digested with NcoI and SmaI. The two restriction endonuclease-digested mixtures were combined

and ligated with T4 DNA ligase (Promega). The nucleotide sequence of the insert in the construct was verified by DNA sequencing.

[0062] Expression and Purification of VrCRP $\Delta$ sp

[0063] A nucleic acid sequence (including SEQ ID NO:3) encoding a signal peptide truncated form of VrCRP, denoted as VrCRP $\Delta$ sp (SEQ ID NO:4 and an N-terminal methionine, and two C-terminal junction residues) was cloned into the pTYB4 expression vector which contains a heterologous bacterial T7 promoter. The resulting construct, pTYB4-VrCRP $\Delta$ sp, was transferred to *E. coli* BL21(DE3). The *E. coli* transformants were cultured in LB (Luria Bertani) liquid medium containing 100  $\mu$ g mL<sup>-1</sup> ampicillin at 37° C. overnight. The overnight culture was diluted to 50-folds with LBA broth and cultured at 37° C. for about 2 h ( $A_{600}$ =0.3 to 0.4). Then culture was gently shaken in an ice bath to bring the temperature down to 24° C. and allowed to grow at 24° C. thereafter. IPTG was added to the culture at a final concentration of 0.3 mM and the culture was incubated at 24° C. for 6 h. The culture was then put into an ice bath for 30 min and the *E. coli* cells were harvested by centrifugation at 4° C., 4400 $\times$ g for 15 min. The expressed VrCRP was purified by intein mediated purification system with an affinity chitin-binding tag according to the method previously described (Chonig et al., 1997, *Gene* 192:271-281). The cells were washed once with distilled water and suspended in a lysis buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, 0.1% TWEEN-20) to obtain a cell suspension ( $A_{600}$ =25 to 30). The cell suspension was homogenized with Microfluidizer and cell debris were removed by centrifugation at 12,000 $\times$ g for 10 min. The supernatant was filtered through a 0.45  $\mu$ m membrane filter. The filtrate containing VrCRP-chitin binding domain (CBD) fusion protein was passed through a chitin affinity column (16 $\times$ 100 mm, bed volume: 30 mL) at a flow rate of 0.4 mL min<sup>-1</sup>. The column was washed with 15-20 fold bed volume of a washing buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, 0.1% TWEEN-20) at a flow rate of 0.9 mL min<sup>-1</sup>. The VrCRP $\Delta$ sp-CBD fusion protein bound to the affinity column was cleaved with DTT by introducing 2.5 fold bed volume of a cleavage buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 30 mM DTT) into the column. The column was then saturated with the cleavage buffer and kept at 4° C. for 16 h. VrCRP $\Delta$ sp was eluted from the column with an elution buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA). VrCRP $\Delta$ sp was further purified with FPLC system using Superdex peptide HR 10/30 column (10 $\times$ 300-310 mm, bed volume 24 mL) (Pharmacia) to remove small amounts of contaminating proteins. The column was equilibrated with 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 10 mM  $\beta$ -mercaptoethanol and VrCRP $\Delta$ sp was eluted with the same buffer at a flow rate of 1.0 mL min<sup>-1</sup>. The purified VrCRP $\Delta$ sp was homogeneous as examined by SDS-PAGE.

[0064] Bioassay of VrCRP $\Delta$ sp Activity

[0065] Activity of VrCRP $\Delta$ sp against *C. chinensis*, one of the major bruchid pests of mung bean, was studied with artificial mung bean seeds. The artificial seeds were prepared according to the method of Shade et al. (1986) *BioTechnology* 12:793-796) with modification. The artificial seeds have an average volume and weight of 95  $\mu$ L and 98.6 mg,

respectively. Each seed is sufficient to hatch six eggs of *C. chinensis*. Thirty artificial seeds were divided into 6 groups for a six-replicate experiment. Ten pairs of *C. chinensis* with equal number of male and female bruchids were introduced into the growth chamber and allowed to lay eggs on the seeds for 24 hours. The number of eggs on the surface of each seed was strictly controlled to six by destroying the extra eggs with a small needle. The seeds were incubated at 25° C. and 60% relative humidity and examined for within-seed development times (WSDT) and percentage emergence at proper time interval. The results were subject to statistic analysis using unpaired student t-test.

[0066] Effect of VrCRPΔsp on the Growth of *Spodoptera frugiperda* Cells

[0067] Fall armyworm (*Spodoptera frugiperda*, Sf 21, Gibco) cells were grown in TNM-FH medium (TNM-FH insect medium, Sigma, T1032) that contained 8% fetal bovine serum (FBS) at 28° C. for 3 days. The cell number was counted under microscope and adjusted to  $6 \times 10^5$  cells per unit. Appropriate amounts of VrCRPΔsp were added and cultured for 3 days. The number of cells was counted under microscope.

[0068] In Vitro Translation Inhibition Assay

[0069] The reaction mixture contained 0.75 μL potassium acetate (1 M), 0.5 μL BMV (Brome Mosaic Virus) RNA (Promega Co.) ( $0.5 \mu\text{g} \mu\text{L}^{-1}$ ), 2 μL unlabeled amino acid mixture except methionine, 1 mM, 1.9 μL L-[<sup>35</sup>S]methionine (500 μCi, Amersham, AG1094), 12.5 μL wheat germ extract (Promega, L4380), 0.5 μL Rnasin ( $40 \text{ U} \mu\text{L}^{-1}$ ) and 10 to 40 [M VrCRPΔsp. The final volume was adjusted to 25 μL by nuclease-free water. The reaction mixture was incubated at 25° C. for 90 min. Aliquots of the reaction mixture were taken, spotted on to a glass-fiber filter and dried at 45° C. for 3 minutes. The filters were dipped in 10% trichloroacetic acid (TCA) then washed thoroughly with 5% TCA, and then finally washed with 95% ethanol and dried. Radioactivity was measured in a liquid scintillation counter.

[0070] Preparation of Antiserum

[0071] Two New Zealand white rabbits were used for raising antibodies against the purified VrCRPΔsp. The purified VrCRPΔsp was covalently coupled to keyhole limpet hemocyanin (KLH) using the bifunctional reagent glutaraldehyde and mixed with complete adjuvant (TiterMax Antiserum Preparation Gold). The rabbits were injected with the antigen-KLH-adjuvant mixture 3 times with a 4-week interval between injections. The amounts of VrCRPΔsp used for the injections were 150, 100 and 50 μg for the first, second and third injection, respectively. The rabbits were bled from carotid artery one month after the final injection. The blood clot was centrifuged for 10 min at 400×g at 4° C. The clear serum was removed and the complement system was inactivated by incubating at 56° C. for 30 min. The serum was lyophilized and stored at -70° C.

[0072] Western Blot Analysis

[0073] Proteins were resolved with 12.5% SDS-PAGE and trans-blotted to a PVDF (Polyvinylidene fluoride) membrane using capillary transfer (Zeng et al., 1999, *Biotechniques* 26:426-430). The membrane was washed with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% TWEEN-20) and equilibrated with TBST containing 1% bovine

serum albumin. The blot on the membrane was treated with anti-VrCRPΔsp antiserum for 90 min and washed. Then treated with the second antibody (anti-rabbit IgG, AP-linked) for another 90 min. After washing the membrane was incubated with the coloring reagent 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium (BCIP/NBT) at 25° C. for 4 min. The reaction was terminated by washing the membrane with distill water for 10 min.

## Results

[0074] Isolation of VrCRP cDNA

[0075] Plant lines which exhibit resistance to pests and pathogens, e.g., to bruchids, are a valuable source for the identification of factors which can confer pest and pathogen resistance. Such plants can be bred to another line of the same species, which is sensitive to the pest. Mendelian analysis of the progeny indicates of the number of heterogeneous genes contributing to resistance. Resistant progeny are identified, e.g., by the bruchid resistance assay described below, and repeatedly bred to create a resistant plant line, nearly isogenic to the sensitive plant line. Thus, one can produce two nearly isogenic lines of the mung bean *Vigna radiata*, such as VC1973A and VC6089A. It was known that VC1973A seeds are susceptible to bruchid *Callosobruchus chinensis*, whereas VC6089A seeds are resistant to bruchids. Feeding tests of *C. chinensis* on the seeds of VC1973A and VC6089A revealed that the developing seeds of VC6089A were bruchid resistant around 15 days after flowering (DAF). The two lines of *Vigna radiata* L. VC1973A and VC6089A were obtained from Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan 741, Taiwan.

[0076] Subsequently, a variety of methods can be used to identify pest-resistant genes. For example, the resistant gene locus can be genetically mapped to a chromosomal locus. Nucleic acid can be isolated from the locus by a variety of techniques, including the use of markers, walking techniques, genomic libraries, and the polymerase chain reaction.

[0077] Alternatively, differential gene expression analysis techniques can be used to identify the resistant alleles. A tissue is identified which exhibits resistance to the pest. mRNA is isolated from the same tissue from isogenic or nearly isogenic, resistant and sensitive plants, and reverse transcribed. The cDNA population unique to the resistant tissue is identified by subtractive hybridization techniques, which are routine for skilled artisans. After subtraction, cDNAs which are expressed in the resistant, but not the sensitive strain are available for amplification, cloning, and sequencing.

[0078] By using suppression subtractive hybridization a full-length cDNA, named VrCRP, was isolated from the bruchid resistant line *Vigna radiata* L. VC6089A. This cDNA (SEQ ID NO:1) encodes a protein of 73 amino acids containing 8 cysteine residues and a signal peptide of 22 amino acids. The sequence of the encoded protein is recited in SEQ ID NO:2. The VrCRP has a cysteine stabilized α-helix motif (CSH motif) similar to a number of cysteine-rich proteins (Kobayashi et al., 1991, *Biopolymer* 31:1213-1220; Froy et al., 1998, *FASEB J* 12:1793-1796) and shows amino acid sequence homology to fabatin-1 and fabatin-2 isolated from fava bean (*Vicia faba*) (47.6% identity)

(Zhang et al., 1997, *FEMS Microbiol. Lett.* 149:59-64),  $\alpha$ -amylase inhibitor from sorghum (*Sorghum bicolor*) (42.6% identity) (Bloch et al., 1991, *FEBS Lett.* 279:101-104) and weak homology (less than 40%) to a number of other small cysteine-rich proteins from a variety of organisms. The VrCRP protein also contains a signal sequence. A variant of the VrCRP protein lacking the signal sequence is designated as VrCRP $\Delta$ sp, and consists of 51 amino acids of VrCRP, commencing with the 23<sup>rd</sup> residue of VrCRP, and terminating at the final residue of VrCRP. This variant has an additional methionine at its N-terminus, and a proline and glycine at its C-terminus.

[0079] Expression of VrCRP in *E. coli*

[0080] *E. coli* M15 transformed with pQE30 harboring full-length VrCRP failed to express VrCRP. The transformants lysed after the IPTG induction (isopropyl  $\beta$ -D-thiogalactopyranoside). When *E. coli* M15 cells were induced at  $A_{600}=0.3$  to 0.5, cell growth was arrested within one hour. Electron microscopic analysis revealed gross morphological aberrations in the induced cells relative to induced cells lacking VrCRP plasmid. The results indicate that VrCRP is toxic to *E. coli* M15. *E. coli* harboring signal peptide truncated VrCRP (denoted as VrCRP $\Delta$ sp) grow normally at an early stage similar to control *E. coli* M15 cells, but their growth slowed down after 1 hour, and stopped after 2 hours. On the other hand, *E. coli* transformants harboring VrCRP failed to grow to an even greater extent.

[0081] Expression was improved by using signal peptide deleted VrCRP in an IMPACT (intein mediated purification with an affinity chitin-binding tag, New England Biolabs, MA) expression system. As described above, VrCRP $\Delta$ sp is a 54 amino acid protein of which 51 amino acids are derived from VrCRP, while the three remaining (methionine, proline, and glycine) are terminal residues resulting from the cloning vector. The system IMPACT was used to express signal peptide truncated VrCRP. The expressed VrCRP $\Delta$ sp fusion protein with a molecular mass of 60.9 kDa was bound to a chitin affinity column. The fusion protein was cleaved with DTT (1,4-dithiothreitol) to give rise to a VrCRP $\Delta$ sp with a molecular mass of 5.9 kDa, and migrates as such on an 4-12% gradient SDS polyacrylamide gel (SDS-PAGE). The eluted VrCRP $\Delta$ sp was further purified to apparent homogeneity by a Superdex Peptide HR 10/30 column, as determined by electrophoresing 4  $\mu$ g of the purified protein on a silver-nitrate stained SDS-PAGE gel. The purified VrCRP $\Delta$ sp was characterized by its molecular mass (5.9 kDa) and N-terminal amino acid sequencing. The purified VrCRP $\Delta$ sp showed molecular mass of 5.9 kDa in SDS-PAGE that corresponds to the calculated molecular weight 5.944 kDa. The forty amino acid N-terminal sequence of VrCRP $\Delta$ sp completely matched to the corresponding nucleotide sequence of VrCRP.

[0082] Bruchid Resistant Activity of VrCRP

[0083] To determine whether VrCRP $\Delta$ sp plays a role in bruchid resistance or not, artificial seeds containing different amounts (0.01%-0.25%, w/w) of the purified VrCRP $\Delta$ sp were produced. The seeds were tested for this resistance to *C. chinensis*. Intact mung bean seeds (TN5, VC1973A and VC6089A) and artificial seeds made from the flour of these three mung bean varieties were also tested for comparison. Bruchids were allowed to lay eggs on the intact and artificial seeds. The number of eggs on the surface of each seed was

confined to six by destroying the extra eggs. The seeds were incubated at 25° C. and 60% relative humidity. Within-seed development times (WSDT) and percentage emergence are used as criteria for bruchid resistance. The results are shown in Table 1:

TABLE 1

| Material Screened                              | Within seed development time | Percentage Emergence |
|------------------------------------------------|------------------------------|----------------------|
| <b>Intact Seeds</b>                            |                              |                      |
| TN5                                            | 26.3 $\pm$ 0.6               | 100 $\pm$ 0          |
| VC1973A                                        | 25.9 $\pm$ 0.9               | 100 $\pm$ 0          |
| VC6089A                                        | —                            | 0 $\pm$ 0            |
| <b>Artificial Seeds with flour:</b>            |                              |                      |
| TN5                                            | 35.4 $\pm$ 1.6               | 51.3 $\pm$ 9.8       |
| VC1973A                                        | 36.4 $\pm$ 1.6               | 53.1 $\pm$ 5.0       |
| VC6089A                                        | —                            | 0 $\pm$ 0            |
| <b>Artificial Seeds with purified protein:</b> |                              |                      |
| VC1973A + 0.01% VrCRP $\Delta$ sp              | 40.7 $\pm$ 2.1               | 12.5 $\pm$ 5.9       |
| VC1973A + 0.09% VrCRP $\Delta$ sp              | 48                           | 2.1 $\pm$ 5.9        |
| VC1973A + 0.20% VrCRP $\Delta$ sp              | —                            | 0 $\pm$ 0            |
| VC1973A + 0.25% VrCRP $\Delta$ sp              | —                            | 0 $\pm$ 0            |
| VC1973A + 0.25% BSA                            | 41.0 $\pm$ 2.6               | 52.6 $\pm$ 7.9       |

[0084] In intact seeds of susceptible mung beans, TN5 and VC1973A, 100% of the eggs hatched to produce adult bruchids after approximately 26 days. However, no adults emerged from eggs on intact VC6089A seeds, the bruchid resistant mung bean line.

[0085] Artificial seeds produced from flour from each respective mung bean line showed similar properties. In artificial seeds of susceptible mung beans, TN5 and VC1973A, greater than 50% of the eggs hatched to produce adult bruchids after approximately 35 days. Again, no adults emerged from artificial eggs made from intact VC6089A seeds, the bruchid resistant mung bean line.

[0086] Artificial seeds were used to test the efficacy of purified recombinant VrCRP $\Delta$ sp. Artificial seed were produced with flour from the susceptible mung bean VC1973A combined with 0.01%, 0.09%, 0.20%, and 0.25% VrCRP $\Delta$ sp. The seeds were compared to artificial seeds with 0.25% bovine serum albumin (BSA). The VrCRP $\Delta$ sp at 0.01% concentration in the artificial seeds reduced the number of emerging bruchids to 12.5% compared to greater than 50% in the BSA control. Moreover, artificial seeds containing 0.2% VrCRP $\Delta$ sp completely arrested bruchid development, as no adults emerged from such seeds. Artificial seeds with VrCRP $\Delta$ sp at this concentration killed bruchid larvae at their first instar stage. Thus, a significant dosage response was observed between the VrCRP $\Delta$ sp concentrations of 0.01%-0.2%. Thus purified recombinant VrCRP $\Delta$ sp polypeptide is a potent antagonist of bruchid development and/or viability.

[0087] The performance of *C. chinensis* larvae reared on the artificial seeds containing various amounts (0.01%-0.25%) of VrCRP $\Delta$ sp was also investigated. Six artificial seeds containing six eggs each were prepared for each dose. Two capsules containing six seeds each were opened after 21, 24, and 27 days respectively. The larvae were counted and weighted. The results were significant, e.g.,  $P < 0.035$  for

control compared to 0.01% VrCRPΔsp at 24 days, and  $P < 0.001$  for 0.01% VrCRPΔsp compared to 0.2% at 21 days. Thus, artificial seeds containing 0.01% VrCRPΔsp significantly retarded larva development. Complete growth arrest was observed with seeds containing either 0.2% or 0.25% VrCRPΔsp (Table 2). These results provide additional evidence to support the toxicity of VrCRPΔsp to *C. chinensis*.

TABLE 2

| Performance of <i>C. chinensis</i> larvae on artificial seeds containing VrCRPΔsp. |                            |                |               |                |
|------------------------------------------------------------------------------------|----------------------------|----------------|---------------|----------------|
|                                                                                    | mean weight of larvae (mg) |                |               |                |
|                                                                                    | Control (0%)               | 0.01% VrCRPΔsp | 0.2% VrCRPΔsp | 0.25% VrCRPΔsp |
| Day 21                                                                             | 5.2                        | 3.1            | 0             | 0              |
| Day 24                                                                             | 6.6                        | 4.6            | 0             | 0              |
| Day 27                                                                             | 8                          | 5.2            | 0             | 0              |

#### [0088] VrCRPΔsp Inhibits Protein Synthesis

[0089] The purified recombinant VrCRPΔsp polypeptide was combined with in vitro translation extracts to determine the effect of the polypeptide on translation. A 40  $\mu$ M concentration of VrCRPΔsp protein completely inhibited the incorporation of amino acids including p355] methionine into acid insoluble fraction. A significant dosage response was observed between 10-40  $\mu$ M of VrCRPΔsp. With 10 LM VrCRPΔsp, a greater than 60% reduction in acid insoluble counts was observed, with 40  $\mu$ M, a greater than 80% reduction. The results indicated that VrCRPΔsp is a strong inhibitor of protein biosynthesis.

#### [0090] Effect of VrCRPΔsp on Growth of *Spodoptera frugiperda* Cells

[0091] VrCRPΔsp was added to the culture medium of an insect cell culture, a culture with cells of the fall armyworm

(*Spodoptera frugiperda*, Sf 21). After VrCRPΔsp addition, cells were incubated at 28° C. and counted after three days. Purified VrCRPΔsp at 3.42  $\mu$ M completely arrested the growth of Sf 21 cells. Rupture of Sf 21 cells under the conditions was observed by phase-contrast microscopy. The concentration of VrCRPΔsp that caused LC<sub>50</sub> (50% lethality concentration) was 1.7  $\mu$ M.

#### [0092] Antifungal Activity of VrCRPΔsp

[0093] Sterile filter discs were prepared containing 0, 1, 2, 3, 6, 10, 20, and 40  $\mu$ g of purified VrCRPΔsp in 20 mM TrisHCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA. The filter discs were placed on agar plates which had been top spread with the fungus *Rhizoctonia solani*. The plates were incubated at 28° C. for 36 to 49 hours. The radius of the zone of inhibition was measured surrounding each disc. A zone of inhibition greater than 2 mm was observed for filter discs containing 10, 20, and 40  $\mu$ g of purified VrCRPΔsp, indicating that at these concentrations, VrCRPΔsp has antifungal activity.

#### [0094] Additional Compositions

[0095] It was described previously that a single dominant bruchid resistance gene (Br) in the wild mung bean accession TC1966 has been transferred a susceptible cultivar and a resistant isogenic (BC<sub>20</sub>F<sub>4</sub>) line was developed by selection for bruchid resistance against the azuki bean weevil. Two novel cyclopeptide alkaloids, denoted vignatic acid A and B were identified in the resistant isogenic line. The addition of vignatic acid A to pellets of mung bean flour at concentrations more than 1% resulted in the complete elimination of the bruchids, but vignatic acid B had no observable effect on bruchids (Sugawara et al, 1996, *J. Agric. Food Chem.* 44:3360-3364; Kaga et al., 1998, *Mol. Gen. Genet.* 258:378-384). However, the insecticidal activity of vignatic acid A alone was unable to explain the resistance of the resistant isogenic line to azuki bean weevil.

[0096] Other embodiments are within the following claims.

#### SEQUENCE LISTING

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ctc ctt ctc gtc tta gcc tct gat gtg gcc gta gag aga gga gag gct      101
Leu Leu Leu Val Leu Ala Ser Asp Val Ala Val Glu Arg Gly Glu Ala
                   15                   20                   25

aga act tgt atg ata aag aaa gaa ggg tgg gga aaa tgc tta att gac      149
Arg Thr Cys Met Ile Lys Lys Glu Gly Trp Gly Lys Cys Leu Ile Asp
                   30                   35                   40

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## -continued

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acc acc tgt gca cat tcg tgc aag aac cgc ggt tac ata ggt gga gat      197
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tgc aaa ggc atg acg cgc acc tgc tat tgc ctc gtc aac tgt      239
Cys Lys Gly Met Thr Arg Thr Cys Tyr Cys Leu Val Asn Cys
   60                65                70

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 20              25              30

Lys Lys Glu Gly Trp Gly Lys Cys Leu Ile Asp Thr Thr Cys Ala His
 35              40              45

Ser Cys Lys Asn Arg Gly Tyr Ile Gly Gly Asp Cys Lys Gly Met Thr
 50              55              60

Arg Thr Cys Tyr Cys Leu Val Asn Cys
 65              70

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 1              5              10              15

aaa tgc tta att gac acc acc tgt gca cat tcg tgc aag aac cgc ggt      96
Lys Cys Leu Ile Asp Thr Thr Cys Ala His Ser Cys Lys Asn Arg Gly
 20              25              30

tac ata ggt gga gat tgc aaa ggc atg acg cgc acc tgc tat tgc ctc      144
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Val Asn Cys
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Lys Cys Leu Ile Asp Thr Thr Cys Ala His Ser Cys Lys Asn Arg Gly
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27

What is claimed is:

1. A purified polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:4, wherein the polypeptide has insecticidal or fungicidal activity.
2. The purified polypeptide of claim 1 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.
3. A composition comprising the polypeptide of claim 1 present in an amount of 0.01% to 10% by weight of composition.
4. The composition of claim 3 wherein the polypeptide is present in an amount of 0.05% to 5% by weight of composition.
5. The polypeptide of claim 1 wherein the amino acid sequence is at least 80% identical to the amino acid sequence of SEQ ID NO:4.
6. The purified polypeptide of claim 5 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.
7. The polypeptide of claim 5 wherein the amino acid sequence is at least 90% identical to the amino acid sequence of SEQ ID NO:2.
8. The purified polypeptide of claim 7 wherein the polypeptide inhibits translation of a messenger RNA in a wheat germ extract.
9. A purified polypeptide comprising the amino acid sequence of SEQ ID NO:4.
10. A purified polypeptide comprising the amino acid sequence of SEQ ID NO:4, with up to 18 conservative amino acid substitutions, wherein the polypeptide has insecticidal or fungicidal activity.

11. A purified polypeptide encoded by a nucleic acid that hybridizes under high stringency conditions to a probe the sequence of which consists of SEQ ID NO: 1, wherein the polypeptide has insecticidal or fungicidal activity.

12. An isolated nucleic acid encoding the polypeptide of claim 1.

13. The isolated nucleic acid of claim 12 further comprising an operably linked heterologous promoter.

14. An isolated nucleic acid encoding the polypeptide of claim 5.

15. The isolated nucleic acid of claim 14 further comprising an operably linked heterologous promoter.

16. An isolated nucleic acid encoding the polypeptide of claim 9.

17. The isolated nucleic acid of claim 16 further comprising an operably linked heterologous promoter.

18. An isolated nucleic acid comprising a strand that hybridizes under high stringency conditions to a single stranded probe, the sequence of which consists of SEQ ID NO:3 or the complement of SEQ ID NO:3.

19. The isolated nucleic acid of claim 18, wherein the nucleic acid encodes a polypeptide that has insecticidal or fungicidal activity.

20. An isolated nucleic acid comprising a strand that hybridizes under high stringency conditions to a single stranded probe, the sequence of which consists of SEQ ID NO: 1 or the complement of SEQ ID NO: 1, wherein the nucleic acid encodes a polypeptide that has insecticidal or fungicidal activity.

\* \* \* \* \*

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TITLE: New mung bean thionin polypeptide and nucleic acid, useful in generating transgenic plants having insecticidal activity against bruchids, such as *Callosobruchus chinensis*, or fungicidal activity against *Rhizoctonia solani*

Basic Abstract Text (6):

ACTIVITY - Insecticide; Fungicide; Pesticide. Sterile filter discs were prepared containing 0, 1, 2, 3, 6, 10, 20 and 40 micro g of purified VrCRP Delta sp (a signal peptide truncated form of mung bean thionin polypeptide) in 20 mM Tris-hydrochloride, 50 mM sodium chloride and 0.1 mM ethylene diamine tetraacetate (EDTA). The filter discs were placed on agar plates which had been top spread with the fungus *Rhizoctonia solani*. The plates were incubated at 28 deg. C for 36-49 hours. A zone of inhibition greater than 2 mm was observed for filter discs containing 10, 20 and 40 micro g of purified VrCRP Delta sp, indicating that at these concentrations, VrCRP Delta sp has anti-fungal activity.

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US 20030091555A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0091555 A1**  
Oita (43) **Pub. Date: May 15, 2003**

(54) **BACTERICIDAL COMPOSITION  
CONTAINING PEPTIDE AND CHELATING  
AGENT**

(30) **Foreign Application Priority Data**

Aug. 22, 2001 (JP) ..... 2001-251048

(75) **Inventor: Shigeru Oita, Zentsuji-shi (JP)**

**Publication Classification**

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**25TH FLOOR**

**NEW YORK, NY 10017-2023 (US)**

(51) **Int. Cl.<sup>7</sup> ..... A61K 38/48; A61K 31/195**

(52) **U.S. Cl. .... 424/94.63; 514/564**

(57) **ABSTRACT**

(73) **Assignee: NATIONAL AGRICULTURAL  
RESEARCH ORGANIZATION,  
Tsukuba-shi (JP)**

A bactericidal composition is provided, which comprises as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin. The bactericidal composition, for example, has the effect of sterilizing food poisoning bacteria at a low concentration and is highly safe.

(21) **Appl. No.: 10/067,124**

(22) **Filed: Feb. 4, 2002**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification <sup>6</sup>:</b><br><b>C12N 15/29, 15/82, C07K 14/415, A01H 5/00</b>                                                                                                                                                                                                                                                                                                                                                                                                                                            | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 96/38562</b><br><b>(43) International Publication Date:</b> 5 December 1996 (05.12.96)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>(21) International Application Number:</b> PCT/US96/08219<br><b>(22) International Filing Date:</b> 31 May 1996 (31.05.96)<br><b>(30) Priority Data:</b><br>08/459,180 2 June 1995 (02.06.95) US<br><b>(71) Applicant:</b> PIONEER HI-BRED INTERNATIONAL, INC.<br>[US/US]; 700 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US).<br><b>(72) Inventor:</b> RAO, Gururaj, A.; 4734 74th Street, Urbandale, IA 50322-1158 (US).<br><b>(74) Agents:</b> SIMON, Soma, G. et al.; 700 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US). |           | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| <b>(54) Title:</b> HIGH THREONINE DERIVATIVES OF $\alpha$ -HORDOTHIONIN<br><br><b>(57) Abstract</b><br><br>Derivatives of $\alpha$ -hordothionin made by position-specific substitution with threonine residues provide threonine in plants.                                                                                                                                                                                                                                                                                                                 |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

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HIGH THREONINE DERIVATIVES OF  $\alpha$ -HORDOTHIONINTECHNICAL FIELD

5        This invention relates to the improvement of feed formulations. Specifically, this invention relates to derivatives of  $\alpha$ -hordothionin which provide higher percentages of threonine in plants.

BACKGROUND OF THE INVENTION

10        Feed formulations are required to provide animals essential nutrients critical to growth. However, crop plants are generally rendered food sources of poor nutritional quality because they contain low proportions of several amino acids which are essential for, but cannot be  
15        synthesized by, animals.

         For many years researchers have attempted to improve the balance of essential amino acids in the proteins of important crops through breeding programs. As more becomes known about storage proteins and the expression of the  
20        genes which encode these proteins, and as transformation systems are developed for a greater variety of plants, molecular approaches for improving the nutritional quality of seed proteins can provide alternatives to the more conventional approaches. Thus, specific amino acid levels  
25        can be enhanced in a given crop via biotechnology.

         One alternative method is to express a heterologous protein of favorable amino acid composition at levels sufficient to obviate food or feed supplementation. For example, a number of seed proteins rich in sulfur amino  
30        acids have been identified. A key to good expression of such proteins involves efficient expression cassettes with seed specific promoters. Not only must the gene-controlling regions direct the synthesis of high levels of mRNA, the mRNA must be translated into stable protein.

35        Among the essential amino acids needed for animal nutrition, often missing from crop plants, are methionine,

threonine and lysine. Attempts to increase the levels of these free amino acids by breeding, mutant selection and/or changing the composition of the storage proteins accumulated in crop plants has met with minimal success.

5 Usually, the expression of the transgenic storage protein was too low. The phaseolin-promoted Brazil nut 2S expression cassette is an example of an effective chimeric seed-specific gene. However, even though Brazil nut protein increases the amount of total methionine and bound

10 methionine, thereby improving nutritional value, there appears to be a threshold limitation as to the total amount of methionine that is accumulated in the seeds. The seeds remain insufficient as sources of methionine.

An alternative to the enhancement of specific amino

15 acid levels by altering the levels of proteins containing the desired amino acid is modification of amino acid biosynthesis. Recombinant DNA and gene transfer technologies have been applied to alter enzyme activity catalyzing key steps in the amino acid biosynthetic

20 pathway. Glassman, U.S. Patent No. 5,258,300; Galili, et al., European Patent Application No. 485970; (1992); incorporated herein in its entirety by reference. However, modification of the amino acid levels in seeds is not always correlated with changes in the level of proteins

25 that incorporate those amino acids. Burrow, et al., Mol. Gen. Genet.; Vol. 241; pp. 431-439; (1993); incorporated herein in its entirety by reference. Although significant increases in free lysine levels in leaves have been obtained by selection for DHDPS mutants or by expressing

30 the E. coli DHDPS in plants, it remains to be shown that these alterations can increase bound target amino acids, which represent some 90% or more of total amino acids. Thus, there is minimal impact on the nutritional value of seeds.

35 Based on the foregoing, there exists a need for methods of increasing the levels of the essential amino acids, threonine, methionine and lysine in seeds of plants.



It is therefore an object of the present invention to provide methods for genetically modifying plants to increase the levels of the essential amino acid threonine in the plants.

5 It is a further object of the present invention to provide seeds for food and/or feed with higher levels of the essential amino acid threonine than wild species of the same seeds.

#### 10 DISCLOSURE OF THE INVENTION

It has now been determined that one class of compounds, the  $\alpha$ -hordothionins, can be modified to enhance their content of threonine.  $\alpha$ -hordothionin is a 45-amino acid protein which has been well characterized. It can be  
15 isolated from seeds of barley (*Hordeum vulgare*). The molecule is stabilized by four disulfide bonds resulting from eight cysteine residues. The amino acid sequence is as provided in SEQUENCE I.D. No.1. In its native form, it is especially rich in arginine and lysine residues,  
20 containing 5 residues (10%) of each. However, it contains only 3 residues (7%) of the essential amino acid threonine.

The protein has been synthesized and the three-dimensional structure determined by computer modeling. The modeling of the protein predicts that the ten charged  
25 residues (arginine at positions 5,10,17,19 and 30, and lysine at positions 1,23,32,38 and 45) all occur on the surface of the molecule. The side chains of the polar amino acids (asparagine at position 11, glutamine at position 22 and threonine at position 41) also occur on the  
30 surface of the molecule. Furthermore, the hydrophobic amino acids (such as the side chains of leucine at positions 8,15 24 and 33 and valine at position 18) are also solvent-accessible.

Three-dimensional modeling of the protein indicates  
35 that the arginine residue at position 10 is critical to retention of the appropriate 3-dimensional structure and

possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A threonine substitution at that point would disrupt the hydrogen bonding involving arginine at position 10, serine at position 2 and lysine at position 45, leading to destabilization of the structure. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provided a protein which folded correctly.

Since threonine is a polar amino acid, the surface polar amino acid residues, asparagine at position 11 and glutamine at position 22, were substituted; and the charged amino acids, lysine at positions 1,23,32 and 38 and arginine at positions 5,17,19, and 30, were substituted with threonine. The resulting compound has the sequence indicated in SEQUENCE I.D. No. 2. The molecule can be synthesized by solid phase peptide synthesis and folds into a stable structure. It has 13 threonine residues (29%).

While SEQUENCE I.D. No. 2 is illustrative of the present invention, it is not intended to be a limitation. Threonine substitutions can also be performed at positions containing charged amino acids. Only arginine at position 10 and lysine at position 45 are critical for maintaining the structure of the protein. One can also substitute at the sites having hydrophobic amino acids. These include positions 8,15,18 and 24. The resulting compound has the sequence indicated in SEQUENCE I.D. NO. 3.

Synthesis of the compounds is performed according to methods of peptide synthesis which are well known in the art and thus constitute no part of this invention. In vitro, the compounds have been synthesized on an applied biosystems model 431a peptide synthesizer using fastmoc™ chemistry involving hbtu [2-(1h-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, as published by Rao, et al., Int. J. Pep. Prot. Res.; Vol. 40; pp. 508-515; (1992); incorporated herein in its entirety by reference.

Peptides were cleaved following standard protocols and purified by reverse phase chromatography using standard methods. The amino acid sequence of each peptide was confirmed by automated edman degradation on an applied biosystems 477a protein sequencer/120a pth analyzer. More preferably, however, the compounds of this invention are synthesized in vivo by bacterial or plant cells which have been transformed by insertion of an expression cassette containing a synthetic gene which when transcribed and translated yields the desired compound. Such empty expression cassettes, providing appropriate regulatory sequences for plant or bacterial expression of the desired sequence, are also well-known, and the nucleotide sequence for the synthetic gene, either RNA or DNA, can readily be derived from the amino acid sequence for the protein using standard reference texts. Preferably, such synthetic genes will employ plant-preferred codons to enhance expression of the desired protein.

#### Industrial Applicability

The following description further exemplifies the compositions of this invention and the methods of making and using them. However, it will be understood that other methods, known by those of ordinary skill in the art to be equivalent, can also be employed.

#### Plants

The genes which code for these compounds can be inserted into an appropriate expression cassette and introduced into cells of a plant species. Thus, an especially preferred embodiment of this method involves inserting into the genome of the plant a DNA sequence coding for a compound of this invention in proper reading frame, together with transcription initiator and promoter sequences active in the plant. Transcription and translation of the DNA sequence under control of the regulatory sequences causes expression of the protein sequence at levels which provide an elevated amount of the protein in the tissues of the plant.

Preferred plants that are to be transformed according to the methods of this invention are cereal crops, including maize, rye, barley, wheat, sorghum, oats, millet, rice, triticale, sunflower, alfalfa, rapeseed and soybean.

5 Synthetic DNA sequences can then be prepared which code for the appropriate sequence of amino acids, and this synthetic DNA sequence can be inserted into an appropriate plant expression cassette.

Likewise, numerous plant expression cassettes and  
10 vectors are well known in the art. By the term "expression cassette" is meant a complete set of control sequences including initiation, promoter and termination sequences which function in a plant cell when they flank a structural gene in the proper reading frame. Expression cassettes  
15 frequently and preferably contain an assortment of restriction sites suitable for cleavage and insertion of any desired structural gene. It is important that the cloned gene have a start codon in the correct reading frame for the structural sequence.

20 In addition, the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high frequency, and a poly-a recognition sequence at the other end for proper processing and transport of the messenger RNA. An  
25 example of such a preferred (empty) expression cassette into which the cDNA of the present invention can be inserted is the pPHI414 plasmid developed by Beach, et al., of Pioneer Hi-Bred International, Inc., Johnston, IA, as disclosed in U.S. patent application No. 07/785,648,  
30 (1991); incorporated herein in its entirety by reference. Highly preferred plant expression cassettes will be designed to include one or more selectable marker genes, such as kanamycin resistance or herbicide tolerance genes.

By the term "vector" herein is meant a DNA sequence  
35 which is able to replicate and express a foreign gene in a host cell. Typically, the vector has one or more endonuclease recognition sites which may be cut in a

predictable fashion by use of the appropriate enzyme such vectors are preferably constructed to include additional structural gene sequences imparting antibiotic or herbicide resistance, which then serve as markers to identify and  
5 separate transformed cells. Preferred markers/selection agents include kanamycin, chlorosulfuron, phosphonothricin, hygromycin and methotrexate. A cell in which the foreign genetic material in a vector is functionally expressed has been "transformed" by the vector and is referred to as a  
10 "transformant."

A particularly preferred vector is a plasmid, by which is meant a circular double-stranded DNA molecule which is not a part of the chromosomes of the cell.

As mentioned above, both genomic and cDNA encoding the  
15 gene of interest may be used in this invention. The vector of interest may also be constructed partially from a cDNA clone and partially from a genomic clone. When the gene of interest has been isolated, genetic constructs are made which contain the necessary regulatory sequences to provide  
20 for efficient expression of the gene in the host cell. According to this invention, the genetic construct will contain (a) a first genetic sequence coding for the protein or trait of interest and (b) one or more regulatory sequences operably linked on either side of the structural  
25 gene of interest. Typically, the regulatory sequences will be selected from the group comprising of promoters and terminators. The regulatory sequences may be from autologous or heterologous sources.

Promoters that may be used in the genetic sequence  
30 include NOS, OCS and CaMV promoters.

An efficient plant promoter that may be used is an overproducing plant promoter. Overproducing plant promoters that may be used in this invention include the promoter of the chlorophyll  $\alpha$ - $\beta$  binding protein and the  
35 promoter of the small sub-unit (ss) of the ribulose-1,5-biphosphate carboxylase from soybean. See e.g. Berry-Lowe, et al., J. Molecular and App. Gen.; Vol. 1; pp. 483-498;

(1982); incorporated herein by reference. These two promoters are known to be light-induced, in eukaryotic plant cells. See e.g., An Agricultural Perspective, A. Cashmore, Pelham, New York; pp. 29-38; (1983); G. Coruzzi, et al., J. Biol. Chem.; Vol. 258; p. 1399; (1983); and P. Dunsmuir, et al., J. Molecular and App. Gen.; Vol. 2; p. 285; (1983); all incorporated herein by reference.

The expression cassette comprising the structural gene for the protein of this invention operably linked to the desired control sequences can be ligated into a suitable cloning vector. In general, plasmid or viral (bacteriophage) vectors containing replication and control sequences derived from species compatible with the host cell are used. The cloning vector will typically carry a replication origin, as well as specific genes that are capable of providing phenotypic selection markers in transformed host cells. Typically, genes conferring resistance to antibiotics or selected herbicides are used. After the genetic material is introduced into the target cells, successfully transformed cells and/or colonies of cells can be isolated by selection on the basis of these markers.

Typically, an intermediate host cell will be used in the practice of this invention to increase the copy number of the cloning vector. With an increased copy number, the vector containing the gene of interest can be isolated in significant quantities for introduction into the desired plant cells. Host cells that can be used in the practice of this invention include prokaryotes, including bacterial hosts such as E. coli, S. typhimurium, and Serratia marcescens. Eukaryotic hosts such as yeast or filamentous fungi may also be used in this invention. Since these hosts are also microorganisms, it will be essential to ensure that plant promoters which do not cause expression of the protein in bacteria are used in the vector.

The isolated cloning vector will then be introduced into the plant cell using any convenient technique,

including electroporation (in protoplasts), retroviruses, bombardment, and microinjection into cells from monocotyledonous or dicotyledonous plants in cell or tissue culture to provide transformed plant cells containing as  
5 foreign DNA at least one copy of the DNA sequence of the plant expression cassette. Preferably, the monocotyledonous species will be selected from maize, sorghum, wheat or rice, and the dicotyledonous species will be selected from soybean, alfalfa, rapeseed, sunflower or tomato. Using  
10 known techniques, protoplasts can be regenerated and cell or tissue culture can be regenerated to form whole fertile plants which carry and express the gene for a protein according to this invention. Accordingly, a highly preferred embodiment of the present invention is a  
15 transformed maize plant, the cells of which contain as foreign DNA at least one copy of the DNA sequence of an expression cassette of this invention.

It will also be appreciated by those of ordinary skill that the plant vectors provided herein can be incorporated  
20 into agrobacterium tumefaciens, which can then be used to transfer the vector into susceptible plant cells, primarily from dicotyledonous species. Thus, this invention provides a method for increasing threonine levels in agrobacterium tumefaciens-susceptible dicotyledonous plants in which the  
25 expression cassette is introduced into the cells by infecting the cells with agrobacterium tumefaciens, a plasmid of which has been modified to include a plant expression cassette of this invention.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Pioneer Hi-Bred International, Inc.
- (ii) TITLE OF INVENTION: High Threonine Derivatives of  
10 Alpha-Hordothionin
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:  
15 (A) ADDRESSEE: Pioneer Hi-Bred International, Inc.  
(B) STREET: 700 Capital Square, 400 Locust Street  
(C) CITY: Des Moines  
(D) STATE: Iowa  
(E) COUNTRY: United States of America  
20 (F) ZIP: 50309
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
25 (D) SOFTWARE: Patent In Release #1.0, Version#1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: PCT  
(B) FILING DATE:  
30 (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Simon, Soma G.  
(B) REGISTRATION NUMBER: 37,444  
35 (C) REFERENCE/DOCKET NUMBER: 354-PCT
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 515-248-4896  
40 (B) TELEFAX: 515-248-4844

## (2) INFORMATION FOR SEQ ID NO:1:

- 45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 45 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Ser | Cys | Cys | Arg | Ser | Thr | Leu | Gly | Arg | Asn | Cys | Tyr | Asn | Leu | Cys |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |
- 55 Arg Val Arg Gly Ala Gln Lys Leu Cys Ala Gly Val Cys Arg Cys Lys



20

25

30

Leu Thr Ser Ser Gly Lys Cys Pro Thr Gly Phe Pro Lys  
 35 40 45

5

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 45 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Ser Cys Cys Thr Ser Thr Leu Gly Arg Thr Cys Tyr Asn Leu Cys  
 1 5 10 15

20

Thr Val Thr Gly Ala Thr Thr Leu Cys Ala Gly Val Cys Thr Cys Thr  
 20 25 30

Leu Thr Ser Ser Gly Thr Cys Pro Thr Gly Phe Pro Lys  
 35 40 45

25

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 45 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Thr Ser Cys Cys Thr Ser Thr Thr Gly Lys Thr Cys Tyr Asn Thr Cys  
 1 5 10 15

40

Thr Thr Thr Arg Ala Thr Thr Thr Cys Ala Gly Val Cys Thr Cys Thr  
 20 25 30

Leu Thr Ser Ser Gly Thr Cys Pro Thr Gly Phe Pro Lys  
 35 40 45

## WHAT IS CLAIMED IS:

1.

A protein having the sequence of SEQUENCE I.D. No. 3  
5 wherein the amino acid residues at one or more of positions  
1,5,7,8,11,15,17,18,19,22,23,24,30,32,34,38 and 41 are  
threonine, and the remainder of the residues at those  
positions are the residues at the corresponding positions  
in SEQUENCE I.D. No. 1.

10

2.

The protein of Claim 1 wherein one or more of the  
amino acid residues at 1,5,7,11,17,19,22,23,30,32,34,38 and  
41 are threonine.

3.

15

The protein of Claim 2 wherein at least 5 of the amino  
acid residues at positions 1,5,7,11,17,19,22,23,30,32,34,38  
and 41 are threonine.

4.

The protein of Claim 3 wherein at least 7 of the amino  
20 acid residues at positions 1,5,7,11,17,19,22,23,30,32,34,38  
and 41 are threonine.

5.

A nucleotide sequence which codes for a protein having  
the sequence of SEQUENCE I.D. No. 3 wherein the amino acid  
25 residues at one or more of positions  
1,5,7,8,11,15,17,18,19,22,23,24,30, 32,34,38 and 41 are  
threonine, and the remainder of the residues at those  
positions are the residues at the corresponding positions  
in SEQUENCE I.D. No. 1.

30

6.

An RNA sequence which codes for a protein having the  
sequence of SEQUENCE I.D. No. 3 wherein the amino acid  
residues at one or more of positions  
1,5,7,8,11,15,17,18,19,22,23,24,30, 32,34,38 and 41 are  
35 threonine, and the remainder of the residues at those  
positions are the residues at the corresponding positions  
in SEQUENCE I.D. No. 1.

7.

A DNA sequence which codes for a protein having the sequence of SEQUENCE I.D. No. 3 wherein the amino acid residues at one or more of positions  
5 1,5,7,8,11,15,17,18,19,22,23,24,30,32,34,38 and 41 are threonine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE I.D. No. 1.

8.

10 An expression cassette containing the DNA sequence of Claim 7 operably linked to plant regulatory sequences which cause the expression of the DNA sequence in plant cells.

9.

15 A bacterial transformation vector comprising an expression cassette according to Claim 8, operably linked to bacterial expression regulatory sequences which cause replication of the expression cassette in bacterial cells.

10.

20 Bacterial cells containing as a foreign plasmid at least one copy of a bacterial transformation vector according to Claim 9.

11.

Transformed plant cells containing at least one copy of the expression cassette according to Claim 8.

25 12.

The transformed plant cells of Claim 11, wherein the cells are of a monocotyledonous species.

13.

30 The transformed plant cells of Claim 12, wherein the cells are selected from the group consisting of maize, sorghum, wheat and rice cells.

14.

The transformed cells of Claim 11, wherein the cells are of a dicotyledonous species.

15.

The transformed cells of Claim 14, wherein cells are selected from the group consisting of soybean, alfalfa, rapeseed, sunflower, tobacco and tomato cells.

5

16.

A maize cell or tissue culture comprising cells according to Claim 13.

17.

10 A method for enhancing the threonine content of a plant cell or seed comprising the step of expressing a protein according to Claim 1 in the cell or seed.

18.

The method of Claim 17 wherein the plant is a dicotyledonous plant.

15

19.

The method of Claim 17 wherein the plant is a monocotyledonous plant.

20.

20 The method of Claim 19 wherein the threonine content of the plant seed is enhanced.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/08219

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/82 C07K14/415 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                           | Relevant to claim No. |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | WO,A,94 16078 (PIONEER HI BRED INT) 21<br>July 1994                                                                                                                                                                                                                                                                          | 1,2,5-16              |
| Y          | see the whole document                                                                                                                                                                                                                                                                                                       | 1,2,5-20              |
| Y          | THE PLANT JOURNAL,<br>vol. 3 , no. 5, 1993,<br>pages 721-727, XP002015695<br>KARCHI, H., ET AL.: "Seed-specific<br>expression of a bacterial desensitized<br>aspartate kinase increases the production<br>of seed threonine and methionine in<br>transgenic tobacco"<br>see page 726, left-hand column, line 33 -<br>line 37 | 1,2,5-20              |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

14 October 1996

Date of mailing of the international search report

28.10.96

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## INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/08219

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                          | Relevant to claim No. |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | PROTEIN ENGINEERING,<br>vol. 7, no. 12, 1 December 1994,<br>pages 1485-1493, XP000482988<br>RAO A G ET AL: "STRUCTURE-FUNCTION<br>VALIDATION OF HIGH LYSINE ANALOGS OF<br>-HORDOTHIONIN DESIGNED BY PROTEIN<br>MODELING"                                                                                                                                    | 1,2,5-7               |
| A          | see the whole document<br>---                                                                                                                                                                                                                                                                                                                               | 3,4,8-20              |
| A          | PLANT MOLECULAR BIOLOGY,<br>vol. 24, 1994,<br>pages 83-96, XP002015812<br>FLORACK, D.E.A., ET AL.: "Expression of<br>biologically active hordothionins in<br>tobacco. Effects of pre- and pro-sequences<br>at the amino and carboxyl termini of the<br>hordothionin precursor on mature protein<br>expression and sorting"<br>see the whole document<br>--- | 8-16                  |
| A          | WO,A,94 10315 (PIONEER HI BRED INT) 11 May<br>1994<br>see page 14, line 1 - line 14; claim 1<br>---                                                                                                                                                                                                                                                         | 1-20                  |
| A          | PROCEEDINGS OF THE NATIONAL ACADEMY OF<br>SCIENCES OF USA,<br>vol. 91, March 1994, WASHINGTON US,<br>pages 2577-2581, XP002015697<br>KARCHI, H., ET AL.: "Lysine synthesis and<br>catabolism are coordinately regulated<br>during tobacco seed development"<br>see page 2581, left-hand column, last line<br>- right-hand column<br>---                     | 1-20                  |
| A          | WO,A,89 04371 (UNIV LOUISIANA STATE) 18<br>May 1989<br>see page 18, line 25 - page 24, line 10<br>---                                                                                                                                                                                                                                                       | 1-20                  |
| A          | EP,A,0 318 341 (PLANT GENETIC SYSTEMS NV)<br>31 May 1989<br>see page 5, line 60 - page 6, line 57<br>---                                                                                                                                                                                                                                                    | 1-20                  |
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| A          | WO,A,93 03160 (DU PONT) 18 February 1993<br>see the whole document<br>-----                                                                                                                                                                                                                                                                                 | 1-20                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/08219

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)                                                                                                                                                                                                               | Publication<br>date                                                                                                                                                  |
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| WO-A-9416078                              | 21-07-94            | AU-A- 6162294<br>CA-A- 2161881                                                                                                                                                                                                           | 15-08-94<br>21-07-94                                                                                                                                                 |
| WO-A-9410315                              | 11-05-94            | AU-A- 5446694                                                                                                                                                                                                                            | 24-05-94                                                                                                                                                             |
| WO-A-8904371                              | 18-05-89            | AU-A- 2802989<br>CA-A- 1321157<br>EP-A- 0675960                                                                                                                                                                                          | 01-06-89<br>10-08-93<br>11-10-95                                                                                                                                     |
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